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(54) Title: METHODS AND REAGENTS TO DETECT AND CHARACTERIZE NORWALK AND RELATED VIRUSES

(57) Abstract

Double-stranded cDNA was synthesized from nucleic acid extracted from Norwalk virus purified from stool specimens of volunteers. Single-stranded RNA probes derived from the DNA clone after subcloning into an in vitro transcription vector were also used to show that the Norwalk virus contains an ssRNA genome of about 8 kb in size. The availability of a Norwalk-specific cDNA and the genome sequence information allow rapid cloning of the entire genome and establishment of sensitive diagnostic assays. Such assays can be based on detection of Norwalk and Norwalk-related virus nucleic acids or Norwalk and Norwalk-related viral antigens using probes or primers and polyclonal or monoclonal antibodies to proteins expressed from the cDNA or to synthetic peptides made based on the knowledge of the genome sequence. Assays using proteins deduced from the Norwalk virus genome and produced in expression systems can measure antibody responses. Vaccines for Norwalk and related viruses are made from an expressed Norwalk virus protein.

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                          21                              41
G TGC TCT GGG AGC GGG CAT ACA GGT TGG TGG CGA CAG GCC CTC CAA
  cys ser gly ser gly his thr gly trp trp arg gln ala leu gln

              61                              81
  AGC CAA AGG TAT CAA CAA AAT TTG CAA CTG CAA GAA AAT TCT TTT
  ser gln arg tyr gln gln asn leu gln leu gln glu asn ser phe

          101                             121
  AAA CAT GAC AGG GAA ATG ATT GGG TAT CAG GTT GAA GCT TCA AAT
  lys his asp arg glu met ile gly tyr gln val glu ala ser asn

      141                         161                         18
  CAA TTA TTG GCT AAA AAT TTG GCA ACT AGA TAT TCA CTC CTC CGT
  gln leu leu ala lys asn leu ala thr arg tyr ser leu leu arg

  1                       201                             221
  GCT GGG GGT TTG ACC AGT GCT GAT GCA GCA AGA TCT GTG GCA GGA
  ala gly gly leu thr ser ala asp ala ala arg ser val ala gly

                  241                             261
  GCT CCA GTC ACC CGC ATT GTA GAT TGG AAT GGC GTG AGA GTG TCT
  ala pro val thr arg ile val asp trp asn gly val arg val ser

              281                             301
  GCT CCC GAG TCC TCT GCT ACC ACA TTG AGA TCC GGT GGC TTC ATG
  ala pro glu ser ser ala thr thr leu arg ser gly gly phe met

      321                         341                         36
  TCA GTT CCC ATA CCA TTT GCC TCT AAG CAA AAA CAG GTT CAA TCA
  ser val pro ile pro phe ala ser lys gln lys gln val gln ser

  1                       381                             401
  TCT GGT ATT AGT AAT CCA AAT TAT TCC CCT TCA TCC ATT TCT CGA
  ser gly ile ser asn pro asn tyr ser pro ser ser ile ser arg

                  421                             441
  ACC ACT AGT TGG GTC GAG TCA CAA AAC TCA TCG AGA TTT GGA AAT
  thr thr ser trp val glu ser gln asn ser ser arg phe gly asn

              461                             481
  CTT TCT CCA TAC CAC GCG GAG GCT CTC AAT ACA GTG TGG TTG ACT
  leu ser pro tyr his ala glu ala leu asn thr val trp leu thr

      501
  CCA CCC GGT TCA ACC
  pro pro gly ser thr
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Methods and Reagents To Detect and Characterize Norwalk and Related Viruses

This application is a Continuation-in-Part of Applicant's Co-Pending U.S. Application Serial No. 07/443,492 filed November 8, 1989, U.S. Application Serial No. 07/515,993, now abandoned, filed April 27, 1990, U.S. Application Serial No. 07/573,509 filed August 27, 1990, and U. S. Application Serial No. 07/696,454 filed May 6, 1991, all entitled "Methods and Reagents To Detect and Characterize Norwalk and Related Viruses."

This invention is supported in part through grants or awards from the Food and Drug Administration and the National Institute of Health. The United States Government may have certain rights to this invention.

Field of the Invention

The present invention relates generally to synthesizing clones of Norwalk virus and calicivirus and to making probes to Norwalk and related viruses. It also relates to methods of detection and characterization of Norwalk and related viruses.

Background of the Invention

Norwalk virus is one of the most important viral pathogens causing acute gastroenteritis, the second most common illness in the United States (Dingle et al., *Am. J. Hyg.* 58:16-30 (1953); Kapikian and Chanock, "Norwalk group of viruses" in B.N. Fields' 2d ed. of Virology, Raven Press, New York, pp. 671-693 (1990)). Up to 42% of cases of adult viral gastroenteritis have been estimated to be caused by Norwalk or Norwalk-like viruses (Kaplan et al., *Ann. Internal Med.* 96(6):756-761 (1982)). Both water and foodborne transmission of Norwalk virus has been documented, and particularly large epidemic outbreaks of illness have occurred following consumption of contaminated shellfish, including clams, cockles, and oysters (Murphy et al., *Med. J. Aust.* 2:329-333 (1979);

Gunn et al., *Am. J. Epidemiol.* 115:348-351 (1982); Wilson et al., *Am. J. Public Health* 72:72-74 (1982); Gill et al., *Br. Med. J.* 287:1532-1534 (1983); DuPont *New Engl. J. Med.* 314:707-708 (1986); Morse et al., *New Engl. J. Med.* 314:678-681 (1986); Sekine et al., *Microbiol. Immunol.* 33:207-217 (1989)). An increase in fish and shellfish-related food poisonings has recently been noted and attributed to increased recognition of these entities by clinicians as well as to increased consumption of seafood (Eastaugh and Shepherd, *Arch. Intern. Med.* 149:1735-1740 (1989)).

Norwalk virus was discovered in 1973. Until recently, knowledge about the virus has remained limited because it has failed to grow in cell cultures and no suitable animal models have been found for virus cultivation. Human stool samples obtained from outbreaks and from human volunteer studies, therefore, are the only source of the virus. Still, the concentration of the virus in stool is usually so low that virus detection with routine electron microscopy is not possible (Dolin et al., *Proc. Soc. Exp. Med. and Biol.* 140:578-583 (1972); Kapikian et al., *J. Virol.* 10:1075-1081 (1972); Thornhill et al., *J. Infect. Dis.* 132:28-34 (1975)). Current methods of Norwalk virus detection include immune electron microscopy and other immunologic methods such as radio immunoassays (RIAs) or a biotin-avidin enzyme linked immunoabsorbent assays (ELISAs) which utilize acute and convalescent phase serum from humans. To date, no hyperimmune antiserum from animals has been successfully prepared due either to insufficient quantities or unusual properties of the viral antigen. Preliminary biophysical characterization of virions has indicated particles contain one polypeptide (Greenberg et al., *J. Virol.* 37: 994-999 (1981)), but efforts to characterize the viral genome have failed.

Viruses related to Norwalk virus include small round enteric viruses, such as viruses with typical calicivirus morphology and the astroviruses. The classification scheme for the human small enteric viruses shown in Table 1 here is an updated version of a scheme outlined

by Caul and Appleton in the *Journal of Medical Virology*, 9:257-265 (1982). This system is referred to in Cubitt *et al.*, *J. Infectious Diseases*, 156:806-814 (1987); Table 1 of the article by Appleton entitled "Small round viruses: classification and role in food-borne infections", in the book Novel Diarrhoea Viruses, Ciba Foundation Symposium No. 128, pp. 108-125 (John Wiley & Sons, N.Y. (1987)); and Table 1 of the chapter entitled "Norwalk group of viruses" by Kapikian and Chanock from the book Virology (B.N.Fields, 2d ed., Raven Press (1990)).

As shown in Table 1, human small round structured enteric viruses include calicivirus and astrovirus. The recent sequencing of Norwalk virus indicates that Norwalk virus is a calicivirus and has a genome organization like that of other caliciviruses. In addition to the human small round enteric viruses are a large number of non-human small round viruses which have been classified as astroviruses, caliciviruses, and small round structured viruses based upon their morphology. Examples of these viruses are the primate calicivirus isolated from the pygmy chimpanzee, described in the journal *Science* 221:79-81 (1983), a porcine enteric calicivirus, described in the *Journal of Clinical Microbiology* 12:105-111 (1980), and bovine astroviruses described in *Vet Pathol.* 21:208-215 (1984). Individual calicivirus types will at times exhibit host specificity and tissue tropisms, but as an overall group they cause gastroenteritis, hepatitis, abortion, skin lesions, pneumonia, myocarditis, and encephalitis. The caliciviruses infecting humans fit in this context in that Norwalk-like viruses cause gastroenteritis, hepatitis E causes hepatitis, and San Miguel sea lion virus type 5 causes skin vesicles in humans as well as infections in seals, fish, pigs and cattle. (D. O. Matson "Calicivirus Infections" in *Textbook of Pediatric Infectious Disease*, 3d ed., R. D. Feigin and J. D. Cherry, eds., W. B. Sanders, Philadelphia, (in press)).

Summary of the Invention

It is therefore an object of the invention to detect and characterize the Norwalk and related virus genomes by synthesizing and cloning a cDNA library.

It is an associated object of the invention to deduce amino acid sequences from Norwalk and related viral cDNA.

Another object of the invention is to develop probes or primers to confirm the genetic relationship between the Norwalk virus and the Norwalk-related viruses.

Still another object of the invention is to develop a method of preparing polyclonal and monoclonal antibodies to the Norwalk and related viruses.

Yet still another object of the invention is to develop a method of making probes to detect Norwalk and related viruses.

A further object of the invention is to use the cDNA or fragments or derivatives thereof in assays to detect Norwalk and related viruses in samples suspected of containing the viruses.

A still further object of the invention is to express proteins to measure antibody responses.

A nucleotide sequence of the genome sense strand of the Norwalk virus cDNA clone intended to accomplish the foregoing objects includes the nucleotide sequence shown in Table 2. Within the Norwalk nucleotide sequence are regions which encode proteins. The nucleotide sequence of the Norwalk virus genome, its fragments and derivatives are used to make diagnostic products, vaccines and antivirals.

Other and still further objects, features and advantages of the present invention will be apparent from the following description of a presently preferred embodiment of the invention.

Brief Description of the Figures

Figure 1. EM picture of Norwalk and related viruses. Norwalk virus (A),

human Calicivirus (B), small round structured virus (C), and human astrovirus (D). The var is 0.1 μm.

Figure 2a. Hybridization of stool samples with ^{32}P-labeled plasmid DNA for screening positive Norwalk cDNA clones. Nucleic acids from paired stools [before (b) and after (a) infection with Norwalk virus] from two volunteers (1 and 2) were dotted on Zetabind filters. Replicate strips were prepared and hybridized at 50°C and 65°C with each test clone (pUC-27, pUC-593, pUC-13 and pUCNV-953). One clone (pUCNV-953) which reacted only with stool samples after (but not before) Norwalk infection was considered as a potential positive clone and was chosen for further characterization.

Figure 2b. Dot blot hybridization of clone ^{32}P-labeled pUCNV-953 with another 3 sets of stool samples collected at different times after infection (B = before acute phase of illness; A = acute phase of illness; P = post-acute phase of illness) of 3 volunteers. The nucleic acids were dotted directly or after treatment with RNAse or with DNAse before dotting. Double-stranded homologous cDNA (pUCNV-953) was dotted after the same treatments as the stool samples.

Figure 3. Dot blot hybridization of Norwalk viruses in a CsCl gradient with ssRNA probes made from pGEMNV-953. Aliquots of 50ul from each fraction in a CsCl gradient were dotted onto a Zetabind filter. Duplicates of filters were made and hybridized with the two ssRNA probes respectively. The two strands were subsequently called cRNA (positive hybridization with the viral nucleic acid) and vRNA (no hybridization with the viral nucleic acid, data not shown). The graph shows EM counts of Norwalk viruses from each fraction of the same CsCl gradient for the dot blot hybridization. Five squares from each grid were counted and the average of the number of viral particles per square was calculated.

Figure 4. The nucleotide sequence of the genome sense strand of the first Norwalk virus cDNA clone. The deduced amino acid sequence of a long open reading frame in this cDNA also is shown.

Figure 5. Schematic diagram of Norwalk cDNA clones. pUCNV-953 was the first positive cone identified. Overlapping clones were determined by restriction enzyme analyses and partial sequencing of the clones. AAA indicates the poly(a) tail at the 3' end of the viral genome.

Figure 6. Norwalk virus encodes an RNA-directed RNA polymerase sequence motif. The deduced amino acid sequence of a portion of Norwalk virus pUCNV-4095 (NV) is compared with consensus amino acid residues thought to encode putative RNA-directed RNA polymerases of hepatitis E virus (HEV), hepatitis C virus (HCV), hepatitis A virus (HAV), Japanese encephalitis virus (JE), poliovirus (polio), foot-and-mouth disease virus (FMD), encephalomyocarditis virus (EMC), Sindbis virus (SNBV), tobacco mosaic virus (TMV), alfalfa mosaic virus (AMV), brome mosaic virus (BMV), and cowpea mosaic virus (CpMV). Sequences for viruses other than NV are from Figure 3 of Reyes et al., Science 247:1335-1339 (1990).

Figure 7. Three pairs of initial primers used to amplify the Norwalk virus genome. RNA was extracted from a stool sample (sample 543-11) by the CTAB technique and amplified by RT-PCR. Lanes 1 and 5, 1-kb markers from Bethesda Research Laboratories (the markers that migrated as 1.6, 1.0 and 0.5 kb are labeled); lane 2, PCR with Norwalk virus primers 8 and 9; lane 3, PCR with Norwalk primers 16 and 17; lane 4, PCR with Norwalk primers 1 and 4. The amplified products were separated on the agarose gel and visualized with UV light after staining with ethidium bromide. The small product seen in lane 3 was made in variable amount in different experiments. The positions of the three primer pairs used in this study are given above the autoradiograph. The numbers below the map indicate the size (in base pairs) of the RT-PCR product.

Figure 8. This schematic shows the organization of Norwalk genome given in Table 2. The features shown here are based on analyses of the nucleotide sequence of the Norwalk virus genome and the deduced amino acid sequence of proteins encoded in the genome. The genome contains 7753 nucleotides including 111 A's at the 3'-end. Translation of the sequence predicts that the genome encodes three open reading frames (shown by the open boxes in the second line). The first open reading frame is predicted to start from an initiation codon at nucleotide 146 and it extends to nucleotide 5359 (excluding the termination codon). The second open reading frame is initiated at nucleotide 5346 and it extends to nucleotide 6935, and a third open reading frame exists between nucleotides 6938 and 7573. Based on comparisons of these predicted proteins with other proteins in the protein databank, the first open reading frame is a protein that is eventually cleaved to make at least three proteins. These three proteins include a picornavirus 2C-like protein, a 3C-like protease and a 3D-like RNA-dependent RNA polymerase. The second open reading frame encodes the capsid protein, which contains sequence homology with the picornavirus VP3 protein.

Figure 9. Nucleotide and amino acid sequence of human calicivirus Sapporo cDNAs. The 551 nucleotide known sequence of human calicivirus Sapporo (HuCV Sapporo) is presented in its entirety. Below the nucleotide sequence is the amino acid sequence for HuCV Sapporo. Above the HuCV Sapporo nucleotide sequence is the sequence of the cDNA from a Houston day care center outbreak (Day care). In the Day care sequence a "." indicates the nucleotide is identical to the HuCV Sapporo nucleotide at that site. Where a nucleotide difference occurred in the Day care sequence, a new letter is indicated at that position. "N" indicates uncertainty of the nucleotide at that site. Below the HuCV Sapporo amino acid sequence are arrows indicating the extent of cDNAs at23s2m31 and c-29_4-gel (which together contribute to the 551 nucleotides of the known sequence) and the new 36 primer (see Table 6).

Figure 10. Nucleotide homologies between calicivirus cDNAs and calicivirus strains with known sequences. All comparisons are in reference to the sequence of human calicivirus Sapporo. The length of the baseline indicates the known sequence region. The boxes indicate areas of nucleotide sequence homology between HuCV Sapporo and the indicated strain. The length of the box indicates the part of the indicated strain where homology exists and the height of the box indicates the strength of the homology. SD = standard deviation. SD 3 or greater is significant. The numbers under the Norwalk homology box indicate the region of the Norwalk virus genome where homology was observed.

Figure 11. Strategy used to obtain nucleotide sequence of the Norwalk-related virus SRSV/KY/89 using primers from the Norwalk virus sequence. This figure shows a partial schematic of the Norwalk virus genome and the predicted ORF1 showing the location of the 3D-like polymerase region, the second ORF showing the location of the VP3-like domain and the start of ORF 3. On the bottom, the solid lines show regions of KY89 sequenced based on using primer sets (see numbers such as 36 and 35, etc) chosen from the sequence of the Norwalk virus genome.

Figure 12. Comparison of the Norwalk virus nucleotide sequence with the Norwalk virus-related virus SRSV/KY/89 nucleotide sequence. Part of the nucleotide sequence of Norwalk-related virus SRSV/KY/89 was determined using primers from the Norwalk-virus (NV) genome. Primers from the NV genome used to obtain the sequence of this Norwalk-related virus are shown in Table 6. Some of these primers were modified based on the initial nucleotide sequence obtained from the SRSV/KY/89 to obtain the rest of the sequence of SRSV/KY/89. The primers shown here and in Table 6 are used by way of example only; other NV primers can be used.

Figure 13. Comparison of deduced amino acid sequence of proteins of the Norwalk virus and the Norwalk-related virus SRSV/KY/89. The protein

sequence of SRSV/KY/89 was deduced from the nucleotide sequence shown in Figure 12. Figure 13a shows a comparison of the deduced amino acid sequence of ORF2, the capsid, of SRSV/KY/89 with the same region encoded in the Norwalk virus genome. Figure 13b shows a comparison of the deduced amino acid sequence of part of the polymerase protein of SRSV/KY/89 with that of Norwalk virus. Comparisons of similar sequences from other Norwalk-related viruses will permit discovery of conserved and divergent regions including antigenic regions. The information will rapidly permit choices of broadly reactive primers to detect all Norwalk-related viruses and specific primer sets to detect individual Norwalk-related viruses. Similarily, fragments and peptides with common amino acid sequences or specific amino acid sequences can be selected for development of diagnostics, vaccines and antivirals.

Figure 14. Comparison of partial nucleotide sequences of Norwalk virus and six Norwalk-related viruses obtained using primers from the NV genome. Sequences from SRSV/CDC 6/91, SRSV/UT/88, SMA/78; SRSV/Cambridge, UK/92, SRSV/CDC 32, Norwalk virus/68, SRSV-3/88, SRSV/KY89/89. Figures 14a and 14b show two different regions of the genome.

Figure 15. Expression of the Norwalk virus capsid protein. Baculovirus recombinants (C-6 and C-8) that contain a subgenomic piece of Norwalk virus DNA (from nucleotides 5337 to 7753) were selected and used to infect insect *(Spodoptera fugiperda)* cells at a multiplicity of infection of 10 PFU/cell. After 4 days of incubation at 27°C, the infected cells were harvested and the proteins were analyzed by electrophoresis on 12% polyacrylamide gels. The proteins were visualized after staining with Coomassie blue. The Norwalk-expressed protein (highlighted by the arrowhead) is only seen in the recombinant-infected cells, but not in wild-type baculovirus (wt) or mock-infected (m) insect cells.

Figure 16. The Norwalk virus expressed protein shows immunoreactivity with sera from volunteers infected with Norwalk virus. The expressed protein shown in Figure 11 was absorbed onto the wells of a 96-well ELISA plate and its reactivity was tested with dilutions of serum samples taken from volunteers before (pre) and three weeks after (post) infection with Norwalk virus. After an incubation at 37°C for 2 hours, a peroxidase-conjugated goat-anti-human IgG, IgM and IgA serum was added and reactivity was subsequently observed by reading the optical density at 414nm after addition of the substrate. The data show that post-infection sera reacted strongly with the expressed antigen at serum dilutions of 1:100 and 1:1000, and some sera were still specifically reactive at a dilution of 1:10,000.

Figure 17. Baculovirus recombinants containing the 3'-end of the Norwalk genome produce virus-like particles in insect cells. Lysates from insect cells infected with baculovirus recombinant C-8 (see Figure 11) were analyzed by electron microscopy and shown to contain numerous virus-like particles. These particles are the same size as virus particles obtained from the stools of volunteers infected with Norwalk virus. Bar = 50 nm.

Figure 18. Norwalk virus-like particles can be purified in gradients of CsCl. Supernatants of insect cells infected with the baculovirus recombinant C-8 were processed by extraction with genetron and PEG precipitation and virus eluted from these PEG pellets was centrifuged in CsCl gradient in a SW50.1 rotor for 24 hours at 4°C. The gradient was fractionated and material in each fraction was adsorbed onto two wells of an ELISA plate. Duplicate wells were then treated either with pre- or post-infection serum, peroxidase-conjugated goat anti-human serum and substrate and the reactions were monitored by reading the OD_{414nm}. A peak was observed in the gradient at a density of 1.31 g/cm^3 and this peak was shown to contain virus-like particles by electron microscopy. This peak also contained a major protein of an approximate molecular weight

of 58,500 that co-migrated with the protein expressed in the insect cells from the same baculovirus recombinant.

Figure 19. Use of the expressed virus-like particles to measure the reactivity of pre- and post-serum samples from volunteers infected with Norwalk virus shows that most volunteers have an immune response. Volunteer 6 who did not show an immune response also did not become ill after being administered virus.

Figure 20. Partial sequence of the primate Pan paniscus cDNA atprcvw2.

Detailed Description of the Invention

It is readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The term "fragment" as used herein is defined as any portion of the Norwalk virus genome or a subgenomic clone of the Norwalk virus that is required to be expressed to produce or encodes a peptide which in turn is able to induce a polyclonal or monoclonal antibody. It is possible a peptide of only 5 amino acids could be immunogenic but usually peptides of 15 amino acids or longer are required. This depends on the properties of the peptide and it cannot be predicted in advance.

The term "derivative" as used herein is defined as larger pieces of DNA or an additional cDNA which represents the Norwalk virus genome and which is detected by direct or sequential use of the original cDNA and any deduced amino acid sequences thereof. Clone pUCNV-1011, therefore, is a derivative, although it does not overlap or share sequences with the original clone. Also included within the definition of derivative are RNA counterparts of DNA fragments and DNA or cDNA fragments in which one or more bases have been substituted or to which labels and end structures have been added without affecting the reading or expression of the DNA or cDNA.

The terms Norwalk "related viruses" and "Norwalk-like viruses" as used herein are defined as human and non-human calicivirus, astrovirus and small round structured viruses (SRSV). As the genomic sequences of most of these viruses are not known, this classification is based on morphology as described by Caul and Appleton in the *Journal of Medical Virology*, 9:257-265 (1982); by Appleton in the article entitled "Small round viruses: classification and role in food-borne infections", in the book Novel Diarrhoea Viruses, Ciba Foundation Symposium No. 128, pp. 108-125 (John Wiley & Sons, N.Y. (1987)); and by Kapikian and Chanock in the chapter entitled "Norwalk group of viruses" from the book Virology (B.N.Fields, 2d ed., Raven Press (1990)). As the genomic sequences of the viruses become known, those skilled in the art will be able to determine Norwalk-related viruses and Norwalk-like viruses based on nucleotide homologies.

Within the Norwalk-related viruses is a subgroup of viruses referred to herein as the SRSV's or the Norwalk group. The Norwalk group includes Snow Mountain Agent (SMA), Hawaii Agent, Taunton Agent, Amulree, Otofuke, and Montgomery County Agent. The Norwalk group is characterized by small, round, structured viruses with an amorphous surface or ragged outline.

Production of Norwalk Virus for Molecular Cloning

Norwalk virus was produced by administration of safety tested Norwalk virus (8FIIa) to adult volunteers. The virus inoculum used in the volunteer study, was kindly supplied by Dr. Albert Kapikian (Laboratory of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). This virus originated from an outbreak of acute gastroenteritis in Norwalk, Ohio (Dolin et al., 1971). Two ml of a 1 to 100 dilution of 8FIIa in TBS was administered orally to each individual with 80 ml of milli-Q water (Millipore, Bedford, MA 01730). Sodium bicarbonate solution was taken by each person 2 minutes before and 5 minutes after virus administration. The volunteer studies were approved by the Institutional

Review Board for Human Research at Baylor College of Medicine, at the Methodist Hospital and at the General Clinical Research Center. The virus was administered to the volunteers in the General Clinical Research Center where the volunteers were hospitalized and under extensive medical care for 4 days. All stools were collected and kept at -70°C for later use.

Purification of Norwalk Viruses from Stool Samples

A 10% solution of stool samples in TBS was clarified by low speed centrifugation at 3000 rpm for 15 minutes. The resulting supernate then was extracted two to three times with genetron in the presence of 0.5% Zwittergent 3-14 detergent (Calbiochem Corp., La Jolla, CA). Viruses in the aqueous phase were concentrated by pelleting at 36,000 rpm for 90 minutes through a 40% sucrose cushion in a 50.2 Ti rotor (Beckman Instruments, Inc., Palo Alto, CA 94304). The pellets were suspended in TBS and mixed with CsCl solution (refractive index 1.368) and centrifuged at about 35,000 rpm for about 24 hours in a SW50.1 rotor (Beckman). The CsCl gradient was fractionated by bottom puncture and each fraction was monitored for virus by EM examination. The peak fractions containing Norwalk virus were pooled and CsCl in the samples was diluted with TBS and removed by pelleting the viruses at about 35,000 rpm for 1 hour. The purified virus was stored at about -70°C.

Extraction of Nucleic Acids from Purified Virus

One method of extraction involved treating purified Norwalk virus from CsCl gradients with proteinase K (400 ug/ml) in proteinase K buffer (0.1 M Tris-Cl pH 7.5, 12.5 mM EDTA, 0.15 M NaCl, 1% w/v SDS) at about 37°C for about 30 minutes. The samples were then extracted once with phenol-chloroform and once with chloroform. Nucleic acids in the aqueous phase were concentrated by precipitation with 2.5 volumes of ethanol in the presence of 0.2 M NaOAc followed by pelleting for 15 minutes in a microcentrifuge.

cDNA Synthesis and Cloning of Amplified of cDNA

One method of synthesis and cloning included denaturing nucleic acids extracted from the purified Norwalk viruses with 10 mM CH_3HgOH. Then cDNA was synthesized using the cDNA synthesis kit with the supplied random hexanucleotide primer (Amersham, Arlington Heights, IL 60005). After the second strand synthesis, the reaction mixture was extracted once with phenol-chloroform and once with chloroform followed by ethanol precipitation. Amplification of DNA was performed using the random prime kit for DNA labeling (Promega Corp., Madison, WI 53711-5305). Eight cycles of denaturation (100°C for 2 minutes), reannealing (2 minutes cooling to room temperature) and elongation (room temperature for 30 minutes) were performed after addition of Klenow fragment (Promega Corp.). A DNA library was constructed in pUC-13 with blunt-end ligation into the Sma I site.

Screening of the Library for Positive Clones

As one method of screening, white colonies from transformed DH5 alpha bacterial cells (BRL) were picked and both a master plate and minipreps of plasmid DNA were prepared for each clone. Clones containing inserts were identified after electrophoresis of the plasmid DNA in an agarose gel. The insert DNA in the agarose gel was cut out and labeled with ^{32}P using random primers and Klenow DNA polymerase such as in the PRIME-A-GENE® labeling system (Promega Corp.). Other isotopic or biochemical labels, such as enzymes, and fluorescent, chemiluminescent or bioluminescent substrates can also be used. Nucleic acids extracted from paired stool samples (before and after Norwalk infection) from two volunteers (543 and 544) were dotted onto Zetabind filters (AFM, Cuno, Meriden, CT). Replicate filter strips were prepared and hybridized with each labeled plasmid probe individually at 65°C without formamide. Potential positive clones were judged by their different reactions with the pre- and post-infection stools. Clones which reacted with post (but not pre-) infection stools of volunteers were considered positive and these clones on the master plates were

characterized further. Once one Norwalk clone was identified, it was used to rescreen the cDNA library to identify additional overlapping clones. Rescreening the cDNA library with these additional clones can ultimately identify clones representing the entire Norwalk virus genome.

<u>Reverse Transcriptase-Polymerase Chain Reaction Production of cDNA Clones from Viruses Related to Norwalk Virus</u>

One method for producing cDNA clones of viruses related to Norwalk virus using the knowledge of the Norwalk virus genome sequence is the reverse transcription-polymerase chain reaction method. In this procedure, RNA was extracted from 300 uL of specimen containing the related virus. Complementary DNA was prepared by reverse transcriptase-polymerase chain reaction (RT-PCR) using a primer pair (for example primers 36 and 35 shown in Table 6) derived from the sequence of Norwalk virus. The resulting product was ligated into a plasmid vector and transfected into *E. coli*. Plasmids then were partially purified from the bacteria and the inserted PCR product was sequenced in the plasmid by dideoxy chain termination to examine the relation to Norwalk virus by nucleotide and predicted protein homology.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1

<u>Electron micrograph confirmation</u>

To permit better diagnosis and molecular characterization of Norwalk virus and related viruses, a cDNA library for Norwalk was derived from nucleic acid extracted from virions purified from stool samples. Norwalk virus was purified with methods used previously for hepatitis A and rotaviruses from stool samples with some modifications (Jiang et al., 1986). Basically, stool samples obtained from volunteers administered Norwalk virus were treated with genetron to remove lipid and water insoluble materials. Virus in the aqueous phase was then pelleted through a 40% sucrose cushion. The resulting pellets were

resuspended, sonicated and loaded in a CsCl gradient for isopycnic centrifugation.

Figure 1 shows an electron micrographs of purified Norwalk viruses isolated by the above procedure and Norwalk-related viruses used to produce cDNAs using RT-PCR.

Example 2

Initial cDNA synthesis, cloning and screening

A cDNA library was generated from nucleic acids extracted from these purified viruses by proteinase K treatment of the samples followed by phenol-chloroform extraction and ethanol precipitation (Jiang et al., 1986; 1987). Because the nature of the viral genome was unknown, the extracted nucleic acids were denatured with methylmercuric hydroxide before cDNA synthesis. Random primed cDNA was synthesized with the Gubler-Hoffman method (cDNA synthesis system plus, Amersham) and a small amount of cDNA was obtained. Direct cloning of this small amount of cDNA was unsuccessful. Therefore, a step of amplification of the DNA was performed by synthesizing more copies of the DNA with random primers and the Klenow fragment of DNA polymerase before cloning. The procedure involved cycles of denaturation, addition of random primers and the Klenow fragment of DNA polymerase, reannealing and elongation. With this procedure, a linear incorporation of labeled nucleotides into product was observed as the number of cycles of synthesis was increased. The number of cycles performed was limited (<10) to avoid the synthesis of an excess of smaller fragments. In the case of Norwalk cDNA, eight cycles of amplification were performed and approximately 2.5 ug of DNA were obtained, which was at least a 100-fold amplification of the starting template cDNA. This amplified cDNA was cloned into pUC-13 by blunt-end ligation and a positive clone (pUCNV-953) was isolated.

To obtain the positive Norwalk virus clone, minipreparations of the plasmid DNAs containing potential inserts were screened by agarose gel electrophoresis. Inserts of the larger clones in the gel were cut out and probes were made with the DNA in the gel using the PRIME-A-GENE®

labeling system (Promega Corp.). These probes were hybridized individually with paired stool samples (before and after Norwalk infection) from two volunteers (Figure 2a). One clone (pUCNV-953) reacted with post- but not pre-infection stool samples from both volunteers.

Example 3

Confirmation of viral origin of the clone pUCNV-953

To further confirm the viral origin of the clone pUCNV-953, six more paired stool samples were tested and the same results were obtained. Figure 2b shows a dot blot hybridization of the clone with stool samples collected at different times post-infection of the disease. Strong signals were observed only with stools from acute phase, but not before and after the illness. This result was consistent with previous RIA assays for viral antigen detection using convalescent sera from volunteers with Norwalk diarrhea and immune electron microscopy (IEM) studies of the samples for viral particle examination. This result also agrees with the patterns of virus shedding in stool in the course of the disease (Thornhill et al., 1975). When the pUCNV-953 clone was hybridized with fractions of a CsCl gradient from the Norwalk virus purification scheme, an excellent correlation between hybridization and EM viral particle counts was observed (Figure 3). The peaks of the hybridization signals and viral particle counts both were at fractions with a density of 1.38 g/cm^3, which agrees with previous reports of the biophysical properties of Norwalk virus. Finally, the clone was tested by hybridization with highly purified Norwalk virus electrophoresed on an agarose gel. A single hybridization band was observed with Norwalk virus but not with HAV and rotavirus. Sequence analysis of the pUCNV-953 cDNA showed this clone is 511 bp (Figure 4). This partial genomic cDNA encodes a potential open reading frame for which the amino acid sequence has been deduced (Figure 4). No significant nucleotide or deduced amino acid sequence homology was found by comparison with other sequences in the Gen Bank (Molecular Biology Information Resource, Eugene Software, Baylor College of Medicine).

Example 4

Use of Norwalk virus cDNA to characterize the viral genome

The pUCNV-953 cDNA was subcloned into the transcription vector pGEM-3Zf(+) and grown. ssRNA probes were then generated by in vitro transcription using SP6 and T7 polymerases (Bethesda Research Laboratory). When two opposite sense ssRNA probes were hybridized with the viral nucleic acid separately, only one strand reacted with the virus, indicating the viral genome is single-stranded. As shown in Figure 2b, the hybridization signals were removed by treatment of the viral nucleic acid with RNAse (but not with DNAse) before loading them onto the filters, indicating the virus genome contains ssRNA. A long open reading frame was found in one of the two strands of the inserted DNA by the computer analysis of the sequences of pUCNV-953. The ssRNA probe with the same sequence as this coding strand does not react with the viral nucleic acid, but the complementary ssRNA probe does react in the hybridization tests. Therefore, Norwalk virus contains a positive sense single-stranded RNA genome. The size of the genome of Norwalk virus was estimated to be about 8 kb based on comparisons of the migration rate of the purified viral RNA in agarose gels with molecular weight markers.

The pUCNV-953 cDNA was used to rescreen a second cDNA library made as follows. A clone of the Norwalk or related virus was synthesized by isolating nucleic acid from purified Norwalk virus; cDNA was synthesized using reverse transcriptase and random primers; a second strand of DNA was synthesized from the cDNA; and at least one copy of DNA was inserted into a plasmid or a cloning and expression vector; and screening the library with the original puCNV-953 cDNA identified clones containing fragments of (or the complete) Norwalk or related genome. Alternatively at least one copy of DNA was inserted in a cloning and expression vector, such as lambda ZAPII® (Stratagene Inc.), and the cDNA library was screened to identify recombinant phage containing fragments of or the complete Norwalk or related genome. Additional cDNAs were made and found with this method. Use of these additional cDNAs to

made and found with this method. Use of these additional cDNAs to rescreen the library resulted in detection of new clones (Figure 5).

Thus, those skilled in the art will recognize that entire Norwalk virus cDNA sequence, or fragments or derivatives thereof, can be used in assays to detect the genome of Norwalk and other related viruses. The detection assays include labeled cDNA or ssRNA probes for direct detection of the Norwalk virus genome and measurement of the amount of probe binding. Alternatively, primers or small oligonucleotide probes (10 nucleotides or greater) and polymerase chain reaction amplification are used to detect the Norwalk and Norwalk-related virus genomes. Expression of the open reading frame in the cDNA is used to make hyperimmune or monoclonal antibodies for use in diagnostic products, vaccines and antivirals.

Using the above methodology, the nucleotide sequence in Table 2 was identified. Within that nucleotide sequence, the encoding regions for several proteins have been identified. In that sequence, the first protein is encoded by nucleotides 146 through 5339 and the amino acid sequence is shown in Table 3. This first protein is eventually cleaved to make at least three proteins including a picornavirus 2C-like protein, a 3C-like protease and an RNA-dependent RNA polymerase. The RNA-dependent RNA polymerase is deduced from nucleotides 4543 to 4924 of the Norwalk virus genome as shown in Table 3. The fact that this portion of the genome contains an RNA polymerase is verified by comparisons with RNA polymerase in other positive sense RNA viruses (Figure 6 SEQ ID NOS 38 through 50).

Also in the sequence in Table 2, two other protein encoding regions were found. They are encoded by nucleotides 5346 through 6935 and nucleotides 6938 through 7573. The amino acid sequences for these two proteins are shown in Tables 4 and 5, respectively.

Example 5

Diagnostic assays based on detection of the sequences of the Norwalk virus genome

Hybridization assays are the assays of choice to detect Norwalk virus because small amounts of virus are present in clinical or contaminated water and food specimens. Previously, detection of Norwalk and related nucleic acids was not possible because the genome of Norwalk virus was not known and no sequence information was available. Probes made from the Norwalk virus cDNA or primers made from the Norwalk virus genome sequence allow methods to amplify the genome for diagnostic products to be established. Probes to identify Norwalk virus alone and to identify other Norwalk-related viruses enable development of either specific assays for Norwalk or general assays to detect sequences common to many or all of the Norwalk-related agents.

In the past, one major difficulty encountered in RT-PCR detection of viral RNA in stool samples was that uncharacterized factor(s) are present in stools which inhibit the enzymatic activity of both reverse transcriptase and Taq polymerase (Wilde et al., *J. Clin. Microbiol.* 28:1300-1307, 1990). These factor(s) were difficult to remove by routine methods of nucleic acid extraction. Techniques were developed using cetyltrimethylammonium bromide (CTAB) and oligo d(T) cellulose specifically to separate viral RNA from the inhibitory factor(s). These techniques were based on the unique properties of CTAB which selectively precipitates nucleic acid while leaving acid insoluble polysaccharide in the supernatant. The resulting nucleic acid was further purified by adsorption onto and elution from oligo d(T) cellulose. This step removes unrelated nucleic acids that lack a poly(A) tail. With this technique, Norwalk virus was detected easily by PCR in very small amounts (400 ul of a 10% suspension) of stool sample. For example, one skilled in the art will recognize that it is now possible to clone the genome of RNA viruses present in low concentrations in small amounts of stool after RT-PCR and a step of amplification of the viral RNA by RT-PCR using random primers. In some cases, RT-PCR active nucleic acids are extracted with

CTAB and without oligo d(T) cellulose. In addition, now that the inhibitor(s) can be removed from stool, it is also possible to detect and clone nucleic acids of other viruses (DNA viruses, non-poly(A) tailed RNA viruses) present in stool.

The CTAB and oligo d(T) cellulose technique of extraction followed by detection of viral RNA with RT-PCR was used on stool samples and could be used on water and food samples. Stool sample was suspended in distilled water (about 10% wt/vol) and extracted once with genetron. Viruses in the supernatant were precipitated with polyethylene glycol at a final concentration of about 8%. The viral pellets were treated with proteinase K (about 400 ug/ml) in the presence of SDS at about 37°C for about 30 minutes followed by one extraction with phenol chloroform and one with chloroform. A solution of about 5% CTAB and about 0.4M NaC1 was added at a ratio of sample:CTAB equal to about 5:2. After incubation at about room temperature for about 15 minutes and at about 45°C for about 5 minutes, the nucleic acids (including the viral RNA) were collected by centrifugation in a microcentrifuge for about 30 minutes. The resultant pellets were suspended in about 1M NaC1 and extracted twice with chloroform. The viral RNA in the aqueous phase was used directly in RT-PCR reactions or further purified by adsorption/elution on oligo d(T) cellulose.

A batch method of adsorption/elution on oligo d(T) cellulose was used to purify poly(A) tailed RNA. In this procedure, nucleic acids partially purified as described above or RNA extracted directly with phenol chloroform (without CTAB treatment) were mixed with oligo d(T) cellulose (about 2-4mg/sample) in a binding buffer (about 0.5M NaC1 and 10mM Tris, pH 7.5). The mixture was incubated at about 4°C for about 1 hr with gentle shaking and then centrifuged for about 2 minutes in a microcentrifuge. The oligo d(T) cellulose pellet was washed 3-4 times with binding buffer and then the poly(A) tailed RNA was eluted with 1X TE buffer (about 10mM Tris, 1mM EDTA, pH 7.5). The supernate was collected following centrifugation to remove the oligo d(T) cellulose and the viral RNA in the supernate was precipitated with ethanol. The RNA

obtained at this stage was basically inhibitor-free and able to be used in RT-PCR.

In preliminary experiments, Norwalk virus RNA was detected in less than 0.05g of stool samples using the CTAB technique. A trace inhibitor activity was observed with RNA extracted with either CTAB or oligo d(T) alone, but this was easily removed by dilution (1:2) of the extracted nucleic acid before RT-PCR. Combination of the CTAB and oligo d(T) techniques resulted in obtaining high quality, inhibitor free RNA which could be used directly for RT-PCR detection and for cloning of the viral genome. With development of this method to clone from small amounts of stool, one skilled in the art will know that they can obtain cDNAs for the remainder of the genome including those representing the 5'-end of the genome.

For detection with PCR, primers based on the above nucleotide sequence of the genome were made by chemical methods. These primers include: Primer 1: CACGCGGAGGCTCTCAAT located at nucleotides 7448 to 7465; Primer 4: GGTGGCGAAGCGGCCCTC located at nucleotides 7010 to 7027; Primer 8: TCAGCAGTTATAGATATG located at nucleotides 1409 to 1426; Primer 9: ATGCTATATACATAGGTC located at nucleotides 612 to 629; Primer 16: CAACAGGTACTACGTGAC located at nucleotides 4010 to 4027; and Primer 17: TGTGGCCCAAGATTTGCT located at nucleotides 4654 to 4671 (SEQ ID NOS 51 through 56, respectively). These primers have been shown to be useful to detect virus using reverse transcription and polymerase chain reaction methods (RT-PCR). Figure 7 shows data using these primers. In primer sets 1 and 4, 8 and 9, and 16 and 17, the reverse compliments for the sequences given above for primers 1, 8, and 17 were used.

New, additional primer sets (Table 6 and SEQ ID NOS.: 15 to 37) are used as probes to detect the Norwalk-related viruses. Table 7 shows the ability of newly selected primer sets 36-35, 69-39, 78-80 to detect many Norwalk-related viruses. These results are additional examples of the use of primer sets from the original Norwalk virus sequence to detect Norwalk-related viruses. Nucleotide sequence data of many of these

viruses indicates that there is a continuum of genetic relatedness within the RNA region described by primer sets 36-35 or 69-39 of these different viruses (from 87% to 0%), yet these different agents can be detected using primers from the Norwalk virus genome sequence. The sequence of 2516 nucleotides of another small round structured virus (SRSV/KY/89 SEQ ID NO:12) also was obtained by using a total of 8 additional sets of primers from the original Norwalk virus sequence (primers 56 and 23, 42 and 55, 58 and 59, 60 and 61, 72 and 63, 76 and 77, 64 and 75, and 74 and 3; Table 6).

Example 6

Preparation of polyclonal antibodies and monoclonal antibodies to Norwalk virus proteins

Protein(s) encoded in the cDNA fragments or derivatives thereof, is produced in a prokaryotic or eukaryotic expression system and used to immunize animals to produce polyclonal antibodies for diagnostic assays. Prokaryotic hosts may include Gram negative as well as Gram positive bacteria, such as E. coli, S. tymphimurium, Serratia marcescens, and Bacillus subtilis. Eukaryotic hosts may include yeast, insect or mammalian cells. Immunized animals may include mammals such as guinea pigs, mice, rabbits, cows, goats or horses or other non-mammalian or non-murine species such as chickens. Repeated immunization of these animals with the expressed protein mixed with an adjuvant such as Freund adjuvant to enhance stimulation of an immune response produces antibodies to the protein.

Alternatively, synthetic peptides of greater than 15 amino acids made to match the amino acid sequence deduced from the partial cDNA sequence (or from other sequences determined by sequencing additional cDNAs detected with the original or other clones) are linked to a carrier protein such as bovine serum albumin or lysozyme or cross-linked with treatment with glutaraldehyde and used to immunize animals to produce polyclonal antibodies for diagnostic tests.

The serum of animals immunized with either the expressed protein or with synthetic peptides are tested by immunologic assays such as immune electron microscopy, Western blots (immunoblots) and blocking ELISAs to demonstrate that antibodies to Norwalk and related viruses have been made. Reactivities with the expressed protein or synthetic peptides show specificity of the polyclonal sera. Reactivities with other viruses in the Norwalk group (Snow Mountain Agent, Hawaii Agent, Taunton Agent, etc.) indicate production of a reagent which recognizes cross-reacting epitopes.

Balb\c mice injected with the immunogens as described above and shown to have produced polyclonal antibodies are boosted with immunogen and then sacrificed. Their spleens are removed for fusion of splenocytes with myeloma cells to produce hybridomas. Hybridomas resulting from this fusion are screened for their reactivity with the expressed protein, the peptide and virus particles to select cells producing monoclonal antibodies to Norwalk virus. Screening of such hybridomas with Norwalk-related viruses permits identification of hybridomas secreting monoclonal antibodies to these viruses as well.

Development of Diagnostic Assays

Analysis of the deduced amino acid sequence of the Norwalk virus genome has shown that the Norwalk virus has the genetic organization shown in Figure 8. Expression of regions of this genome in cell-free translation systems and in the baculovirus expression system have shown that the 5'-end of the genome encodes nonstructural proteins and the 3'-end of the genome encodes at least one structural protein. Based on this information, one can express the complete genome or subgenomic regions of the genome to produce diagnostic assays to detect viral antigens or immune responses to specific regions of the genome. This information can be used to detect the Norwalk virus, antigens or immune responses to Norwalk virus. This information also can be used to detect other similar currently uncharacterized viruses that cause gastroenteritis or possibly other diseases. Some of these viruses will be in the Caliciviridae or in the

picornavirus superfamily. All of these viruses will have matching or similar genomic regions in their DNA sequences.

The availability of cDNA clones from viruses related to Norwalk virus enables the production of new antibodies and antisera for diagnostic assays for these related viruses. For example, availability of cDNA clones from caliciviruses which cannot be cultivated permits the expression of protein products of those clones. The protein products are used to develop new antibodies and antisera. In addition, genetic engineering is used to combine the cDNAs from viruses related to Norwalk virus with the cDNAs from Norwalk virus to produce chimeric proteins, such that part of the protein produced is derived from Norwalk virus genome sequence and another part of the protein is derived from the genome sequence of a virus related to Norwalk virus. These chimeric proteins are then used to produce diagnostic reagents, vaccines and antivirals. Examples of the diagnostic assays are shown in the specific examples and figures below.

Example 7

Development of diagnostic assays to detect nucleic acids of Norwalk virus or Norwalk-related viruses by detection of specific regions of the viral genomes based on an understanding of the Norwalk virus genome.

The genetic organization of the Norwalk virus genome allows the prediction of specific regions of the gene sequence as regions where oligonucleotide primers or probes can be developed to detect Norwalk virus sequences and common sequences of other related or similar viruses. Some of these common genome sequences are found in viruses in the Caliciviridae or in the picornavirus superfamily. The detection can be done by standard PCR, hybridization or other gene amplification methods.

Two primers, named 35 (CTT GTT GGT TTG AGG CCA TAT, complementary to nt 4944-4924 in the Norwalk virus genome, SEQ ID NO: 15) and 36 (ATA AAA GTT GGC ATG AAC A, nt 4475-4493 in the Norwalk virus genome, SEQ ID NO: 16), were chosen from the region likely to encode the Norwalk virus RNA polymerase. These primers then

were used to prepare a cDNA clone by reverse transcriptase-PCR from the nucleotide sequence of human calicivirus Sapporo strain (HuCV Sapporo), 1982 outbreak (Figure 9, SEQ ID NO:5). The resulting sequence was compared to that of Norwalk virus and of feline and rabbit caliciviruses available from Genbank. The first cDNA clone from Sapporo, named "c-29_4-gel", determined to contain calicivirus sequence was 488 nucleotides long, of which 40 nucleotides were contributed by primers 36 and 35, leaving 448 nucleotides unique to human calicivirus Sapporo. The sequence of clone c-29_4-gel between primers 36 and 35 also is shown in Figure 9, SEQ ID NO:8.

Evidence that the HuCV Sapporo cDNA clone was correct is shown by five facts. First, the sequence exhibits strong homology with Norwalk virus, feline calicivirus, and the rabbit calicivirus at the nucleotide and amino acid levels. (See Figure 10 and Tables 7 and 8). Second, the sequence contains a continuous protein encoding region on the positive strand. In Norwalk, feline, and rabbit caliciviruses continuous protein encoding regions also are found in the region of homology. Third, the sequence contains the amino acid motif YGDD, which is a marker for RNA virus proteins which have RNA-dependent-RNA-polymerase activity. In c-29_4-gel, the YGDD motif is at the predicted distance from the ends of the sequence. Fourth, the same cDNA product was obtained from six different stool specimens. Fifth, no significant homologies were found for other sequences in the Genbank.

The nucleotide sequence of c-29_4-gel was used to synthesize an internal primer. This internal primer was used to prepare a second set of RT-PCR products from human calicivirus Sapporo RNA. A number of new cDNA clones were obtained of which one, named "at23s2m31", contains overlapping sequence which is 5' on the virus genome from that contained in c-29_4-gel. Sequence at23s2m31 is 149 nucleotides long (SEQ ID NO:7) and overlaps c-29_4-gel by 46 nucleotides. See Figure 9 for at23s2m31 sequence and area of overlap with c-29_4-gel. The resulting combined sequence information of c-29_4-gel and at23s2m31 is

551 nucleotides in length, excluding the portion c-29_4-gel contributed by prime 35.

Although the human calicivirus Sapporo sequence was generated from knowledge of the Norwalk virus sequence, the former is distinguishable in the same region (see Table 8 or Figure 9). The known sequence of human calicivirus Sapporo indicates that this virus is more closely related to the animal caliciviruses than to Norwalk virus.

In May, 1987, a child in Houston was infected with a virus which was identified as a calicivirus based on its morphology. Samples containing virus particles from this child failed to react in serologic assays developed for the detection of Norwalk virus and human calicivirus Sapporo. Primers 36 and 35 were used to prepare cDNA from the viral genome of this strain using RT-PCR. The resulting cDNA product, called 4847 complete, is 434 nucleotides long, excluding the primers, and is distinguishable from that of Norwalk virus and human calicivirus Sapporo. (See "Houston" in homology comparison in Figure 10; Table 10 and SEQ ID NO:10). Evidence that this Houston cDNA is correct is the same as that listed for c-29_4-gel above, except that homology with Norwalk virus and human calicivirus Sapporo is not statistically significant.

<u>Use of the sequence from the human calicivirus Sapporo strain to produce an amplification primers for human calicivirus Sapporo and related agents</u>

The known sequence of human calicivirus Sapporo overlaps one of the two primers, called primer 36 (see Table 6), used for the initial amplification of cDNA clone c-29_4-gel. Examination of the homology of known calicivirus sequences (Table 8 SEQ ID NOS 57 through 62) in that region indicated that a new 36 primer could be synthesized and used to amplify caliciviruses more closely related to human calicivirus Sapporo than Norwalk virus. A new primer was synthesized and is called primer "new 36" (see Table 6, last line, and SEQ ID NO:37).

The new 36 primer was used with primer 35 to generate a cDNA clone from a calicivirus which caused a diarrhea outbreak in November, 1986, in a Houston day care center ("Day care"). The calicivirus strain causing this Day care outbreak was antigenically related to human calicivirus Sapporo but antigenically distinct from Norwalk virus by EIA. The Day care cDNA product obtained from the RT-PCR reaction with primers new 36 and 35 is 445 nucleotides long, excluding the primers (see Figure 9 and SEQ ID NO:9), and has close homology to human calicivirus Sapporo and a more distant, yet still significant homology with Norwalk virus, as shown in Figure 10. Evidence that this Day care cDNA is correct is the same as that listed for c-29_4-gel above.

Use of primers 35 and 36 derived from the Norwalk virus sequence to derive a cDNA clone from an animal calicivirus

A calicivirus was isolated from the mouth of the pygmy chimpanzee, *Pan paniscus*. This calicivirus is antigenically distinct from the human calicivirus Sapporo strain by EIA. A cDNA was produced from the primate calicivirus (PrCV) RNA using RT-PCR and primers 36 and 35. The complete nucleotide sequence of this cDNA is not yet available. The cDNA, called atprcvw2 (Figure 20; SEQ. ID. NOS 13 and 14), is of the predicted size and has significant nucleotide homology with human calicivirus Sapporo, feline calicivirus(es), and the rabbit calicivirus in the region of known sequence. No significant homology with Norwalk virus has been observed in the region of known sequence. The known amino acid sequence contains the YGDD motif on the positive strand at the predicted distance from primer 35.

Use of multiple primers form the Norwalk virus genomic sequence to detect and characterize KY89, another small round virus associated with an outbreak of gastroenteritis.

The known sequence for Norwalk virus is used to obtain the sequence of other viruses such as SRSV/KY/89, an agent from a stool from an outbreak of gastroenteritis in Japan in 1989. Originally, cDNA

products and sequence information were obtained using primer sets 36-35. Continued work with another 8 sets of primers (Primers 56 and 23, 42 and 55, 58 and 59, 60 and 61, 72 and 63, 76 and 77, 64 and 75, and 74 and 3 in Table 6 and SEQ ID NOS:21 through 36) allowed the SRSV/KY/89 sequence of 2516 nucleotides to be determined (Figures 11 and 12, SEQ ID NO:12). This sequence includes the part of the polymerase region and the capsid region of the genome. Figures 14 and 6 (SEQ ID NOS 38 through 50 and 63 through 75) show sequences from other Norwalk-related viruses. Continued use of this approach with other Norwalk-related viruses (such as those shown in Table 7) allows the discovery of the complete sequences of multiple Norwalk-related viruses. Those skilled in the art will realize that the use of such sequence information and expression of fragments and derivatives of Norwalk-related viruses permits development of diagnostic assays to detect antibodies, antigens, viral genetic material or antivirals and to develop vaccines for specific Norwalk-related viruses in the same manner that Norwalk virus fragments and derivatives have been used.

Example 8

Development of diagnostic assays using expressed Norwalk virus proteins to detect immune responses to Norwalk virus

Protein(s) encoded in the Norwalk virus genome or fragments or derivatives thereof is produced in a prokaryotic or eukaryotic expression system and used as antigens in diagnostic assays to detect immune responses following virus infections. Prokaryotic hosts may include Gram negative as well as Gram positive bacteria, such as *Escherichia coli*, *Salmonella tymphimurium*, *Serratia marcescens*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus sanguinis*. Eukaryotic hosts may include yeast, insect or mammalian cells. Diagnostic assays may include many formats such as enzyme-linked immunosorbent assays, radioimmunoassays, immunoblots or other assays. Figure 15 shows data for a capsid protein encoded from the 3'-end of the Norwalk virus genome. It is expressed by nucleotides 5337 through 7753 of the DNA sequence

shown in Table 2 and Figure 8. This protein has an approximate molecular weight of 58,500 and is hereinafter referred to as the 58,500 mwt protein. It was produced in insect cells infected with baculovirus recombinants (C-6 and C-8). A band (see arrow in Figure 15) representing the 58,500 mwt protein in C-6 and C-8 infected cells is not seen in insect cells infected with wild-type (WT) baculovirus or in mock infected cells. Other proteins encoded by Norwalk virus cDNA or fragments or derivatives are similarly expressed using baculovirus recombinants and other expression systems.

Figure 16 shows data using the 58,500 mwt protein produced using the baculovirus expression system to detect immune responses before and after infection of volunteers with Norwalk virus inoculum. Antigen was put on ELISA plates and pre- and post-infection human sera were added. The data show that when an individual has had the infection, the post-serum reacts strongly to the antigen. Other proteins encoded in the Norwalk virus cDNA or fragments or derivatives thereof are similarly used to detect immune responses following Norwalk virus infection.

Some proteins have the intrinsic property of being able to form particles. The 58,500 mwt protein discussed above has that property. Particles formed from proteins are expressed in any expression system and used to produce diagnostic assays based on detection of antibody responses or immune responses. Figure 17 shows an electron micrograph of particles produced using the baculovirus expression system from recombinants containing the 3'-end of the Norwalk genome. These particles are similar in size to the native virus particles. They are antigenic, immunoreactive and immunogenic. They differ from most of the virus particles resulting from natural infection in that many of the expressed particles lack nucleic acids. The rNV particles are highly immunogenic when given parenterally to mice, rabbits and guinea pigs and when given orally to mice.

Figure 18 shows data on the properties of rNV particles following centrifugation in gradients of CsCl. The density of the particles (symbolized by closed boxes) is 1.31 g/cc which is distinct from the 1.38

g/cc density of particles purified from the original infectious Norwalk inoculum given to volunteers. The gradients were fractionated. Each fraction was put on an ELISA plate and human serum was then introduced. The open boxes show that there was no ELISA activity with the pre-infection serum. The closed diamonds show there was reactivity with the post-infection serum. Other particles made from other proteins encoded in the Norwalk virus cDNA or fragments or derivatives thereof are similarly used to detect immune responses following Norwalk virus infection.

Figure 19 shows data using purified particles formed by the 58,500 mwt protein to detect immune responses in post-inoculation (but not pre-inoculation) serum samples of 9 volunteers infected with Norwalk virus. One of the volunteers, number 6, exhibited no symptoms of Norwalk virus infection based on monitoring clinical symptoms or measuring an immune response. Purified, expressed particles were put on ELISA plates and one pre- and one post-infection serum samples from each volunteer was added to the particles. The amount of antibody binding to the particles in each pre- and post-infection sample was measured. The data in Figure 19 show that the expressed proteins form particles that are immunoreactive and antigenic. Other proteins encoded in the Norwalk virus cDNA or fragments or derivatives thereof are similarly used to detect immunoreactive and antigenic activity.

Additional developments of diagnostic assays for the detection of Norwalk and Norwalk-related viruses also were pursued. First, new ELISA assays were made based on utilizing the Norwalk virus capsid protein that was engineered to be synthesized from a cDNA fragment that was deduced from the Norwalk virus cDNA sequence and then produced using the baculovirus expression system. This expressed Norwalk virus capsid protein self-assembled into recombinant Norwalk virus particles (rNV). Two new ELISA assays were established using this rNV antigen. One assay detects antiviral antibody and the other detects viral antigen. Both the ELISAs are very sensitive when compared to the previous assays (based on reagents from human volunteers) available to detect such

agents. Further characterization of the antibody ELISA has shown this assay detects immune responses following human infections with Norwalk virus and a subset of human infections with viruses in the Norwalk group such as Snow Mountain and Hawaii agents. In contrast, the antigen ELISA is based on use of hyperimmune serum made to the baculovirus expressed recombinant Norwalk virus particles (rNV). This antigen ELISA has been found to be very specific in that is recognizes the prototype Norwalk virus (8FIIa) and a subset of closely related agents, but not all other viruses in the Norwalk group such as the Snow Mountain agent and Hawaii agent (See Tables 1 and 7). While the antigen ELISA does not detect other viruses in the Norwalk group such as the small round structured viruses or caliciviruses, these and other Norwalk-related viruses have been able to be detected using primers selected from the nucleotide sequence of Norwalk virus (See Table 7).

To develop more broadly reactive diagnostic assays, ELISAs based on using other fragments of the Norwalk virus genome were developed. The new diagnostic assays are based on detection of antibody responses or of antigens deduced from fragments of the Norwalk virus genome other than the capsid region. An example and data of this approach is the following.

One Norwalk virus nonstructural protein is predicted to be encoded in the first ORF of Norwalk viral genome. This ORF is located at the 5 end of the viral genome and it has a predicated molecular weight of 190,000 (190K). Whether this ORF 1 is useful in diagnostic assays first was evaluated by expressing the protein encoded in the full length viral RNA, and then synthesizing and testing the immunoreactivity of the encoded protein using a cell-free system. This was accomplished by in vitro transcription of a full length cDNA (pGNV-F) of the Norwalk viral genome cDNAs. This full-length cDNA was constructed by ligation of subgenomic derivatives of the original Norwalk virus cDNAs shown in the physical map in Figure 5. The in vitro synthesized NV mRNAs next were examined for their ability to direct the synthesis of a Norwalk virus specific protein by cell-free translation in rabbit reticulocyte lysates in the

presence of ^{35}S methionine to produce a radiolabeled protein. The expressed proteins were analyzed by polyacrylamide gel electrophoresis (PAGE). A clear band of approximate molecular weight of 130,000 was observed in the sample containing the viral RNA but not in the negative control (without viral RNA). The immunoreactivity of this protein was examined by reactivity with pre- and post-infection sera from volunteers given Norwalk virus. The 130K protein was precipitated by a convalescent serum of a volunteer infected with Norwalk virus, but not by serum collected before infection, indicating this protein was virus-specific. This showed this 130K protein contains some immunoreactive epitopes. The apparent smaller size of the protein made in this translation system suggested that either the protein migrates aberrantly on gels, or an internal initiation codon was used to begin translation or some type of post translational modification may have occurred after the protein was translated.

To further characterize immunoreactive derivatives of the Norwalk virus cDNA useful for diagnostic assays, the 2C region of the Norwalk viral genome (see Figure 8) was expressed using the baculovirus expression system. This region was selected for initial expression because it is located at the 5'-end of the non-structural protein and a high level of conservation was found between the sequence of the predicted Norwalk virus protein, and new sequence published for related caliciviruses and picornavirus. A 5'-end cDNA fragment of the viral genome was subcloned into the baculovirus transfer vector pVL 1393. After co-transfection of insect Sf9 cells with wild-type baculovirus DNA, recombinants containing the Norwalk viral gene were identified and selected. After three rounds of plaque purification, radiolabeled lysates of recombinant-infected insect cells were prepared, and the radiolabeled proteins were analyzed by PAGE. The results showed that a protein of apparent molecular weight of 57,000 (57K) was made in recombinant-infected but not in uninfected cells. The size of the protein suggested that the internal AUG initiation codon located at nucleotide 953 was used for making this protein. This 57K protein also was precipitated by convalescent serum (but not by pre-

infection serum) from a volunteer who was infected with Norwalk virus. This protein mainly remained cell-associated. One skilled in the art will readily see that improvements in the yield and purification of this 2C nonstructural protein are possible and will yield more rapid ELISAs to detect Norwalk and related virus infections. One skilled in the art also will see that by expressing proteins from other regions of the Norwalk viral genome (*e.g.*, 3C-like, 3D-like and the 3d ORF), diagnostic assays are made for Norwalk and related viruses similar to the ELISAs made with the 2C nonstructural and rNV structural protein. These new assays should widen the spectrum in detection of Norwalk-related viruses.

The initial lack of sensitive methods to detect Norwalk and Norwalk-related viruses made the description of the many Norwalk-related viruses difficult to define. However, as shown in Table 7, the methods and data provided here demonstrate how the discovery of the nucleotide sequence of the Norwalk virus genome has led to the ability to develop tests to detect Norwalk virus and other related agents. The data and methods also demonstrate that fragments and derivatives of the Norwalk virus genome can be used to provide evidence of and immunity against Norwalk and related viruses.

Example 9

Development of diagnostic assays using expressed Norwalk virus and Norwalk-related viruses to detect viral antigens

Individual proteins, particles or protein aggregates formed from expression of one or more Norwalk virus genes in any prokaryotic or eukaryotic expression system are used as an immunogen or inoculate animals to produce polyclonal and monoclonal antibodies for diagnostic assays to detect viral antigens.

Recombinant Norwalk virus particles (rNV) produced using the baculovirus expression system has been used to produce polyclonal antibodies in mice, guinea pigs and rabbits following parenteral immunization (see Table 9). Mice given rNV orally also have developed serum antibodies. Hybridomas from mice immunized with rNV also have

been obtain following fusion with myeloma cells. Use of these antibodies in a capture ELISA has shown NV antigen can be detected. This antigen ELISA based on the antiserum made to the rNV particles is quite specific and it detects only a subset of Norwalk-related viruses (See Table 7). Therefore, additional capsid antigens from other Norwalk-related viruses (such a Snow Mountain, Hawaii etc.) must be expressed to produce a more broadly reactive ELISA for capsid antigen. The ELISA is only one format that can be used to detect virus antigen. Other formats could include immunofluorescence or immunocytochemistry, or immune electron microscopy. The comparison of the capsid sequences of Norwalk virus and Norwalk-related viruses permits the identification of conserved regions of the capsid protein and use of fragments of such sequences to immunize animals and can result in the production of antisera with more broad reactivity to Norwalk-related viruses. Alternatively, sequential immunization of animals with expressed proteins of Norwalk and Norwalk-related viruses will result in antiserum with the desired broad reactivity. Antigen detection assays that are specific to one of a few strains of Norwalk and Norwalk-related viruses and additional assays that are more broadly reactive each will have use.

Expression of fragments of proteins encoded in other regions of the genome can be used to produce antiserum to other proteins for use in ELISAs to detect viral antigens. The expression of the first ORF that represents a polyprotein encoded in the 5'-end of the genome and fragment 2C of the polyprotein has shown that each of these nonstructural proteins in immunoreactive and antiserum made to these can be used to develop diagnostic assays to detect these viral proteins. These assays can be broadly reactive and detect many other Norwalk-related viruses because of sequence conservation. Those skilled in the art will recognize that knowledge of the genome organization of Norwalk virus permits similar expression of the same regions of the genomes of other Norwalk-related viruses for use in diagnostic assays to detect viral antigens.

Example 10

Development of a vaccine using

Norwalk virus expressed antigens

Vaccines for Norwalk virus, the Norwalk group of viruses or other small round viruses are made from an expressed Norwalk virus protein. That expressed protein can be a Norwalk virus capsid protein expressed alone or in combination with one or more other Norwalk virus proteins or self-forming particles. For example, the particles shown in Figure 17 were produced using the baculovirus expression system. They are used as a vaccine when expressed alone or in combination with one or more other Norwalk virus proteins. Similarly, the other proteins encoded in the Norwalk virus cDNA or fragments or derivatives thereof are used as a vaccine when expressed alone or in combination with one or more Norwalk virus proteins.

Individuals are vaccinated orally, parenterally or by a combination of both methods. For parenteral vaccination, the expressed protein is mixed with an adjuvant and administered in one or more doses in amounts and at intervals that give maximum immune response and protective immunity. Oral vaccination parallels natural infection by Norwalk virus inoculum, i.e. the individual ingests the vaccine with dechlorinated water or buffer. Oral vaccination may follow sodium bicarbonate treatment to neutralize stomach activity. For example, sodium bicarbonate solution is taken by each person 2 minutes before and 5 minutes after vaccine administration.

Example 11

Production of a vaccine for other agents by using

expressed Norwalk virus capsids as a carrier or vehicle

for the expression of other antigens

or parts of other antigens

Identification of the region of the genome that encodes the Norwalk virus capsid protein and that forms particles following expression (i.e., regions 5346 through 6935 and 5337 through 7753) allows genetic

engineering of the cDNA that encodes the capsid protein to incorporate one or more heterologous pieces of cDNA that encode antigenic epitopes. Expression of such recombinant genes produces a recombinant capsid that is antigenic, induces antibodies, and protects against Norwalk virus and its antigens, and against the heterologous epitopes or antigens.

Alternatively, the Norwalk virus capsid protein carrier is mixed with or covalently linked to one or more heterologous protein antigens or synthetic peptides containing heterologous epitopes. This mixture is antigenic, induces antibodies, and protects against Norwalk virus and its antigens, and against the heterologous epitopes or antigens.

Individuals are vaccinated using the oral and parenteral methods described above in example 10.

Example 12

Kit

Kits for detecting immune responses to Norwalk virus and are prepared by supplying in a container a protein deduced from the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof. Similar proteins are prepared from Norwalk-related viruses to detect immune responses to the Norwalk-related viruses. For example, the protein encoded by Norwalk virus nucleotides 1 through 7753, the protein encoded by Norwalk virus nucleotides 146 through 5359, the protein encoded by Norwalk virus nucleotides 5337 through 7573, the protein encoded by Norwalk virus nucleotides 5346 through 6935, the protein encoded by Norwalk virus nucleotides 6938 through 7573 and any combinations thereof may be used in such kits. The kit can also include controls for false positive and false negatives, reagents and sample collection devices. The kit can be equipped to detect one sample or multiple samples.

Example 13

Kit

Kits for detecting Norwalk viruses and Norwalk-related viruses are prepared by supplying in a container at least one antiserum made from a protein expressed from the deduced amino acid sequence of the Norwalk virus genome shown in Tables 3, 4, or 5 or from a fragment or derivative the deduced amino acid sequence. Similar antiserum are made from proteins encoded by Norwalk-related viruse genomes. For example, an antiserum made to the protein encoded by Norwalk virus nucleotides 1 through 7753, the protein encoded by Norwalk virus nucleotides 146 through 5359, the protein encoded by Norwalk virus nucleotides 5337 through 7573, the protein encoded by Norwalk virus nucleotides 5346 through 6935, the protein encoded by Norwalk virus nucleotides 6938 through 7573 and any combination thereof may be used in such kits. The kit can also include controls for false positives and false negatives, reagents and sample collection devices. The kit can be equipped to detect one sample or multiple samples.

In conclusion, it is seen that the present invention and the embodiments disclosed herein are well adapted to carry out the objectives and attain the ends and advantages mentioned as well as other inherent therein. The novel features characteristic of this invention are set forth in the appended claims. While presently preferred embodiments of the invention have been described for the purpose of disclosure, numerous changes in the details of synthesis and use described herein will be apparent to those skilled in the art. It should be understood, however, that there is no intention to limit the invention to the specific form disclosed, but on the contrary, the intention is to cover all modifications, alternative means of synthesis and use and equivalents falling within the spirit and scope of the invention.

Table 1. Classification of small round viruses.

Featureless viruses [*]

Virus	Physical features	Examples
Enterovirus	RNA BD[#] 1.34 g/cm^3 Size range 20-30 nm	Polio Hepatitis A
Parvovirus	DNA BD 1.38-1.46 g/cm^3 Size range 18-26 nm	Feline/mink/canine Bovine
Candidate parvovirus	DNA? BD 1.38-1.4g/cm^3	Wollan, Ditchling, Parramatta, cockle

Structured viruses [**]

Virus	Morphology	Physical features	Examples
Astrovirus	5--6-pointed surface star	RNA BD 1.36-1.38 g/cm^3 Size range 28-30 nm	Lamb Human
Calicivirus	Surface hollows, ragged outline 'Star of David' configuration	RNA BD 1.36-1.39 g/cm^3 Size range 30-38 nm	Human (Norwalk, UK1-4, and Sapporo and other Japanese strains) Newbury (bovine) Pig
Small round structured virus (SRSV)	Amorphous surface, ragged outline	BD 1.36-1.41 g/cm^3 Size range 30-35 nm	Montgomery County, Hawaii, Taunton, Amulree, Otofuke, Snow Mountain

* Smooth outer edge and no discernible surface structure.
** Surface structure and/or ragged outline
\# BD = buoyant density

Table 2. The nucleotide sequence of Norwalk virus genome.

GGCGTCAAAA	GACGTCGTTC	CTACTGCTGC	TAGCAGTGAA	AATGCTAACA	ACAATAGTAG	60
TATTAAGTCT	CGTCTATTGG	CGAGACTCAA	GGGTTCAGGT	GGGGCTACGT	CCCCACCCAA	120
CTCGATAAAG	ATAACCAACC	AAGATATGGC	TCTGGGGCTG	ATTGGACAGG	TCCCAGCGCC	180
AAAGGCCACA	TCCGTCGATG	TCCCTAAACA	ACAGAGGGAT	AGACCACCAC	GGACTGTTGC	240
CGAAGTTCAA	CAAAATTTGC	GTTGGACTGA	GAGACCACAA	GACCAGAATG	TTAAGACGTG	300
GGATGAGCTT	GACCACACAA	CAAAACAACA	GATACTTGAT	GAACACGCTG	AGTGGTTTGA	360
TGCCGGTGGC	TTAGGTCCAA	GTACACTACC	CACTAGTCAT	GAACGGTACA	CACATGAGAA	420
TGATGAAGGC	CACCAGGTAA	AGTGGTCGGC	TAGGGAAGGT	GTAGACCTTG	GCATATCCGG	480
GCTCACGACG	GTGTCTGGGC	CTGAGTGGAA	TATGTGCCCG	CTACCACCAG	TTGACCAAAG	540
GAGCACGACA	CCTGCAACTG	AGCCCACAAT	TGGTGACATG	ATCGAATTCT	ATGAAGGGCA	600
CATCTATCAT	TATGCTATAT	ACATAGGTCA	AGGCAAGACG	GTGGGTGTAC	ACTCCCCTCA	660
AGCAGCCTTC	TCAATAACGA	GGATCACCAT	ACAGCCCATA	TCAGCTTGGT	GGCGAGTCTG	720
TTATGTCCCA	CAACCAAAAC	AGAGGCTCAC	ATACGACCAA	CTCAAAGAAT	TAGAAAATGA	780
ACCATGGCCG	TATGCCGCAG	TCACGAACAA	CTGCTTCGAA	TTTTGTTGCC	AGGTCATGTG	840
CTTGGAAGAT	ACTTGGTTGC	AAAGGAAGCT	CATCTCCTCT	GGCCGGTTTT	ACCACCCGAC	900
CCAAGATTGG	TCCCGAGACA	CTCCAGAATT	CCAACAAGAC	AGCAAGTTAG	AGATGGTTAG	960
GGATGCAGTG	CTAGCCGCTA	TAAATGGGTT	GGTGTCGCGG	CCATTTAAAG	ATCTTCTGGG	1020
TAAGCTCAAA	CCCTTGAACG	TGCTTAACTT	ACTTTCAAAC	TGTGATTGGA	CGTTCATGGG	1080
GGTCGTGGAG	ATGGTGGTCC	TCCTTTTAGA	ACTCTTTGGA	ATCTTTTGGA	ACCCACCTGA	1140
TGTTTCCAAC	TTTATAGCTT	CACTCCTGCC	AGATTTCCAT	CTACAGGGCC	CCGAGGACCT	1200
TGCCAGGGAT	CTCGTGCCAA	TAGTATTGGG	GGGGATCGGC	TTAGCCATAG	GATTCACCAG	1260
AGACAAGGTA	AGTAAGATGA	TGAAGAATGC	TGTTGATGGA	CTTCGTGCGG	CAACCCAGCT	1320
CGGTCAATAT	GGCCTAGAAA	TATTCTCATT	ACTAAAGAAG	TACTTCTTCG	GTGGTGATCA	1380
AACAGAGAAA	ACCCTAAAAG	ATATTGAGTC	AGCAGTTATA	GATATGGAAG	TACTATCATC	1440
TACATCAGTG	ACTCAGCTCG	TGAGGGACAA	ACAGTCTGCA	CGGGCTTATA	TGGCCATCTT	1500
AGATAATGAA	GAAGAAAAGG	CAAGGAAATT	ATCTGTCAGG	AATGCCGACC	CACACGTAGT	1560
ATCCTCTACC	AATGCTCTCA	TATCCCGGAT	CTCAATGGCT	AGGGCTGCAT	TGGCCAAGGC	1620
TCAAGCTGAA	ATGACCAGCA	GGATGCGTCC	TGTGGTCATT	ATGATGTGTG	GGCCCCCTGG	1680
TATAGGTAAA	ACCAAGGCAG	CAGAACATCT	GGCTAAACGC	CTAGCCAATG	AGATACGGCC	1740
TGGTGGTAAG	GTTGGGCTGG	TCCCACGGGA	GGCAGTGGAT	CATTGGGATG	GATATCACGG	1800
AGAGGAAGTG	ATGCTGTGGG	ACGACTATGG	AATGACAAAG	ATACAGGAAG	ACTGTAATAA	1860
ACTGCAAGCC	ATAGCCGACT	CAGCCCCCCT	AACACTCAAT	TGTGACCGAA	TAGAAAACAA	1920
GGGAATGCAA	TTTGTGTCTG	ATGCTATAGT	CATCACCACC	AATGCTCCTG	GCCCAGCCCC	1980
AGTGGACTTT	GTCAACCTCG	GGCCTGTTTG	CCGAAGGGTG	GACTTCCTTG	TGTATTGCAC	2040
GGCACCTGAA	GTTGAACACA	CGAGGAAAGT	CAGTCCTGGG	GACACAACTG	CACTGAAAGA	2100
CTGCTTCAAG	CCCGATTTCT	CACATCTAAA	AATGGAGTTG	GCTCCCCAAG	GGGGCTTTGA	2160
TAACCAAGGG	AATACCCCGT	TTGGTAAGGG	TGTGATGAAG	CCCACCACCA	TAAACAGGCT	2220
GTTAATCCAG	GCTGTAGCCT	TGACGATGGA	GAGACAGGAT	GAGTTCCAAC	TCCAGGGGCC	2280
TACGTATGAC	TTTGATACTG	ACAGAGTAGC	TGCGTTCACG	AGGATGGCCC	GAGCCAACGG	2340
GTTGGGTCTC	ATATCCATGG	CCTCCCTAGG	CAAAAAGCTA	CGCAGTGTCA	CCACTATTGA	2400
AGGATTAAAG	AATGCTCTAT	CAGGCTATAA	AATATCAAAA	TGCAGTATAC	AATGGCAGTC	2460
AAGGGTGTAC	ATTATAGAAT	CAGATGGTGC	CAGTGTACAA	ATCAAAGAAG	ACAAGCAAGC	2520

Table 2, continued

TTTGACCCCT	CTGCAGCAGA	CAATTAACAC	GGCCTCACTT	GCCATCACTC	GACTCAAAGC	2580
AGCTAGGGCT	GTGGCATACG	CTTCATGTTT	CCAGTCCGCC	ATAACTACCA	TACTACAAAT	2640
GGCGGGATCT	GCGCTCGTTA	TTAATCGAGC	GGTCAAGCGT	ATGTTTGGTA	CCCGTACAGC	2700
AGCCATGGCA	TTAGAAGGAC	CTGGGAAAGA	ACATAATTGC	AGGGTCCATA	AGGCTAAGGA	2760
AGCTGGAAAG	GGGCCCATAG	GTCATGATGA	CATGGTAGAA	AGGTTTGGCC	TATGTGAAAC	2820
TGAAGAGGAG	GAGAGTGAGG	ACCAAATTCA	AATGGTACCA	AGTGATGCCG	TCCCAGAAGG	2880
AAAGAACAAA	GGCAAGACCA	AAAAGGGACG	TGGTCGCAAA	AATAACTATA	ATGCATTCTC	2940
TCGCCGTGGT	CTGAGTGATG	AAGAATATGA	AGAGTACAAA	AAGATCAGAG	AAGAAAAGAA	3000
TGGCAATTAT	AGTATACAAG	AATACTTGGA	GGACCGCCAA	CGATATGAGG	AAGAATTAGC	3060
AGAGGTACAG	GCAGGTGGTG	ATGGTGGCAT	AGGAGAAACT	GAAATGGAAA	TCCGTCACAG	3120
GGTCTTCTAT	AAATCCAAGA	GTAAGAAACA	CCAACAAGAG	CAACGGCGAC	AACTTGGTCT	3180
AGTGACTGGA	TCAGACATCA	GAAAACGTAA	GCCCATTGAC	TGGACCCCGC	CAAAGAATGA	3240
ATGGGCAGAT	GATGACAGAG	AGGTGGATTA	TAATGAAAAG	ATCAATTTTG	AAGCTCCCCC	3300
GACACTATGG	AGCCGAGTCA	CAAAGTTTGG	ATCAGGATGG	GGCTTTTGGG	TCAGCCCGAC	3360
AGTGTTCATC	ACAACCACAC	ATGTAGTGCC	AACTGGTGTG	AAAGAATTCT	TTGGTGAGCC	3420
CCTATCTAGT	ATAGCAATCC	ACCAAGCAGG	TGAGTTCACA	CAATTCAGGT	TCTCAAAGAA	3480
AATGCGCCCT	GACTTGACAG	GTATGGTCCT	TGAAGAAGGT	TGCCCTGAAG	GGACAGTCTG	3540
CTCAGTCCTA	ATTAAACGGG	ATTCGGGTGA	ACTACTTCCG	CTAGCCGTCC	GTATGGGGGC	3600
TATTGCCTCC	ATGAGGATAC	AGGGTCGGCT	TGTCCATGGC	CAATCAGGGA	TGTTACTGAC	3660
AGGGGCCAAT	GCAAAGGGGA	TGGATCTTGG	CACTATACCA	GGAGACTGCG	GGGCACCATA	3720
CGTCCACAAG	CGCGGGAATG	ACTGGGTTGT	GTGTGGAGTC	CACGCTGCAG	CCACAAAGTC	3780
AGGCAACACC	GTGGTCTGCG	CTGTACAGGC	TGGAGAGGGC	GAAACCGCAC	TAGAAGGTGG	3840
AGACAAGGGG	CATTATGCCG	GCCACGAGAT	TGTGAGGTAT	GGAAGTGGCC	CAGCACTGTC	3900
AACTAAAACA	AAATTCTGGA	GGTCCTCCCC	AGAACCACTG	CCCCCCGGAG	TATATGAGCC	3960
AGCATACCTG	GGGGGCAAGG	ACCCCCGTGT	ACAGAATGGC	CCATCCCTAC	AACAGGTACT	4020
ACGTGACCAA	CTGAAACCCT	TTGCGGACCC	CCGCGGCCGC	ATGCCTGAGC	CTGGCCTACT	4080
GGAGGCTGCG	GTTGAGACTG	TAACATCCAT	GTTAGAACAG	ACAATGGATA	CCCCAAGCCC	4140
GTGGTCTTAC	GCTGATGCCT	GCCAATCTCT	TGACAAAACT	ACTAGTTCGG	GGTACCCTCA	4200
CCATAAAAGG	AAGAATGATG	ATTGGAATGG	CACCACCTTC	GTTGGAGAGC	TCGGTGAGCA	4260
AGCTGCACAC	GCCAACAATA	TGTATGAGAA	TGCTAAACAT	ATGAAACCCA	TTTACACTGC	4320
AGCCTTAAAA	GATGAACTAG	TCAAGCCAGA	AAAGATTTAT	CAAAAAGTCA	AGAAGCGTCT	4380
ACTATGGGGC	GCCGATCTCG	GAACAGTGGT	CAGGGCCGCC	CGGGCTTTTG	GCCCATTTTG	4440
TGACGCTATA	AAATCACATG	TCATCAAATT	GCCAATAAAA	GTTGGCATGA	ACACAATAGA	4500
AGATGGCCCC	CTCATCTATG	CTGAGCATGC	TAAATATAAG	AATCATTTTG	ATGCAGATTA	4560
TACAGCATGG	GACTCAACAC	AAAATAGACA	AATTATGACA	GAATCCTTCT	CCATTATGTC	4620
GCGCCTTACG	GCCTCACCAG	AATTGGCCGA	GGTTGTGGCC	CAAGATTTGC	TAGCACCATC	4680
TGAGATGGAT	GTAGGTGATT	ATGTCATCAG	GGTCAAAGAG	GGGCTGCCAT	CTGGATTCCC	4740
ATGTACTTCC	CAGGTGAACA	GCATAAATCA	CTGGATAATT	ACTCTCTGTG	CACTGTCTGA	4800
GGCCACTGGT	TTATCACCTG	ATGTGGTGCA	ATCCATGTCA	TATTTCTCAT	TTTATGGTGA	4860
TGATGAGATT	GTGTCAACTG	ACATAGATTT	TGACCCAGCC	CGCCTCACTC	AAATTCTCAA	4920
GGAATATGGC	CTCAAACCAA	CAAGGCCTGA	CAAAACAGAA	GGACCAATAC	AAGTGAGGAA	4980
AAATGTGGAT	GGACTGGTCT	TCTTGCGGCG	CACCATTTCC	CGTGATGCGG	CAGGGTTCCA	5040
AGGCAGGTTA	GATAGGGCTT	CGATTGAACG	CCAAATCTTC	TGGACCCGCG	GGCCCAATCA	5100

Table 2, continued

TTCAGATCCA	TCAGAGACTC	TAGTGCCACA	CACTCAAAGA	AAAATACAGT	TGATTTCACT	5160
TCTAGGGGAA	GCTTCACTCC	ATGGTGAGAA	ATTTTACAGA	AAGATTTCCA	GCAAGGTCAT	5220
ACATGAAATC	AAGACTGGTG	GATTGGAAAT	GTATGTCCCA	GGATGGCAGG	CCATGTTCCG	5280
CTGGATGCGC	TTCCATGACC	TCGGATTGTG	GACAGGAGAT	CGCGATCTTC	TGCCCGAATT	5340
CGTAAATGAT	GATGGCGTCT	AAGGACGCTA	CATCAAGCGT	GGATGGCGCT	AGTGGCGCTG	5400
GTCAGTTGGT	ACCGGAGGTT	AATGCTTCTG	ACCCTCTTGC	AATGGATCCT	GTAGCAGGTT	5460
CTTCGACAGC	AGTCGCGACT	GCTGGACAAG	TTAATCCTAT	TGATCCCTGG	ATAATTAATA	5520
ATTTTGTGCA	AGCCCCCCAA	GGTGAATTTA	CTATTTCCCC	AAATAATACC	CCCGGTGATG	5580
TTTTGTTTGA	TTTGAGTTTG	GGTCCCCATC	TTAATCCTTT	CTTGCTCCAT	CTATCACAAA	5640
TGTATAATGG	TTGGGTTGGT	AACATGAGAG	TCAGGATTAT	GCTAGCTGGT	AATGCCTTTA	5700
CTGCGGGGAA	GATAATAGTT	TCCTGCATAC	CCCCTGGTTT	TGGTTCACAT	AATCTTACTA	5760
TAGCACAAGC	AACTCTCTTT	CCACATGTGA	TTGCTGATGT	TAGGACTCTA	GACCCCATTG	5820
AGGTGCCTTT	GGAAGATGTT	AGGAATGTTC	TCTTTCATAA	TAATGATAGA	AATCAACAAA	5880
CCATGCGCCT	TGTGTGCATG	CTGTACACCC	CCCTCCGCAC	TGGTGGTGGT	ACTGGTGATT	5940
CTTTTGTAGT	TGCAGGGCGA	GTTATGACTT	GCCCCAGTCC	TGATTTTAAT	TTCTTGTTTT	6000
TAGTCCCTCC	TACGGTGGAG	CAGAAAACCA	GGCCCTTCAC	ACTCCCAAAT	CTGCCATTGA	6060
GTTCTCTGTC	TAACTCACGT	GCCCCTCTCC	CAATCAGTAG	TATGGGCATT	TCCCCAGACA	6120
ATGTCCAGAG	TGTGCAGTTC	CAAAATGGTC	GGTGTACTCT	GGATGGCCGC	CTGGTTGGCA	6180
CCACCCCAGT	TTCATTGTCA	CATGTTGCCA	AGATAAGAGG	GACCTCCAAT	GGCACTGTAA	6240
TCAACCTTAC	TGAATTGGAT	GGCACACCCT	TTCACCCTTT	TGAGGGCCCT	GCCCCCATTG	6300
GGTTTCCAGA	CCTCGGTGGT	TGTGATTGGC	ATATCAATAT	GACACAGTTT	GGCCATTCTA	6360
GCCAGACCCA	GTATGATGTA	GACACCACCC	CTGACACTTT	TGTCCCCCAT	CTTGGTTCAA	6420
TTCAGGCAAA	TGGCATTGGC	AGTGGTAATT	ATGTTGGTGT	TCTTAGCTGG	ATTTCCCCCC	6480
CATCACACCC	GTCTGGCTCC	CAAGTTGACC	TTTGGAAGAT	CCCCAATTAT	GGGTCAAGTA	6540
TTACGGAGGC	AACACATCTA	GCCCCTTCTG	TATACCCCCC	TGGTTTCGGA	GAGGTATTGG	6600
TCTTTTTCAT	GTCAAAAATG	CCAGGTCCTG	GTGCTTATAA	TTTGCCCTGT	CTATTACCAC	6660
AAGAGTACAT	TTCACATCTT	GCTAGTGAAC	AAGCCCCTAC	TGTAGGTGAG	GCTGCCCTGC	6720
TCCACTATGT	TGACCCTGAT	ACCGGTCGGA	ATCTTGGGGA	ATTCAAAGCA	TACCCTGATG	6780
GTTTCCTCAC	TTGTGTCCCC	AATGGGGCTA	GCTCGGGTCC	ACAACAGCTG	CCGATCAATG	6840
GGGTCTTTGT	CTTTGTTTCA	TGGGTGTCCA	GATTTTATCA	ATTAAAGCCT	GTGGGAACTG	6900
CCAGCTCGGC	AAGAGGTAGG	CTTGGTCTGC	GCCGATAATG	GCCCAAGCCA	TAATTGGTGC	6960
AATTGCTGCT	TCCACAGCAG	GTAGTGCTCT	GGGAGCGGGC	ATACAGGTTG	GTGGCGAAGC	7020
GGCCCTCCAA	AGCCAAAGGT	ATCAACAAAA	TTTGCAACTG	CAAGAAAATT	CTTTTAAACA	7080
TGACAGGGAA	ATGATTGGGT	ATCAGGTTGA	AGCTTCAAAT	CAATTATTGG	CTAAAAATTT	7140
GGCAACTAGA	TATTCACTCC	TCCGTGCTGG	GGGTTTGACC	AGTGCTGATG	CAGCAAGATC	7200
TGTGGCAGGA	GCTCCAGTCA	CCCGCATTGT	AGATTGGAAT	GGCGTGAGAG	TGTCTGCTCC	7260
CGAGTCCTCT	GCTACCACAT	TGAGATCCGG	TGGCTTCATG	TGAGTTCCCA	TACCATTTGC	7320
CTCTAAGCAA	AAACAGGTTC	AATCATCTGG	TATTAGTAAT	CCAAATTATT	CCCCTTCATC	7380
CATTTCTCGA	ACCACTAGTT	GGGTCGAGTC	ACAAAACTCA	TCGAGATTTG	GAAATCTTTC	7440
TCCATACCAC	GCGGAGGCTC	TCAATACAGT	GTGGTTGACT	CCACCCGGTT	CAACAGCCTC	7500
TTCTACACTG	TCTTCTGTGC	CACGTGGTTA	TTTCAATACA	GACAGGTTGC	CATTATTCGC	7560
AAATAATAGG	CGATGATGTT	GTAATATGAA	ATGTGGGCAT	CATATTCATT	TAATTAGGTT	7620
TAATTAGGTT	TAATTTGATG	TTAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	7680

Table 2, continued

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	7740
AAAAAAAAAA AAA	7753

Table 3. The amino acid sequence deduced from nucleotides 146 through 5359 of the Norwalk virus genome shown in Table 2.

```
CTCGATAAAG ATAACCAACC AAGAT ATG GCT CTG GGG CTG ATT GGA CAG GTC        172
                            Met Ala Leu Gly Leu Ile Gly Gln Val
                             1               5

CCA GCG CCA AAG GCC ACA TCC GTC GAT GTC CCT AAA CAA CAG AGG GAT        220
Pro Ala Pro Lys Ala Thr Ser Val Asp Val Pro Lys Gln Gln Arg Asp
 10                  15                  20                  25

AGA CCA CCA CGG ACT GTT GCC GAA GTT CAA CAA AAT TTG CGT TGG ACT        268
Arg Pro Pro Arg Thr Val Ala Glu Val Gln Gln Asn Leu Arg Trp Thr
                 30                  35                  40

GAG AGA CCA CAA GAC CAG AAT GTT AAG ACG TGG GAT GAG CTT GAC CAC        316
Glu Arg Pro Gln Asp Gln Asn Val Lys Thr Trp Asp Glu Leu Asp His
             45                  50                  55

ACA ACA AAA CAA CAG ATA CTT GAT GAA CAC GCT GAG TGG TTT GAT GCC        364
Thr Thr Lys Gln Gln Ile Leu Asp Glu His Ala Glu Trp Phe Asp Ala
         60                  65                  70

GGT GGC TTA GGT CCA AGT ACA CTA CCC ACT AGT CAT GAA CGG TAC ACA        412
Gly Gly Leu Gly Pro Ser Thr Leu Pro Thr Ser His Glu Arg Tyr Thr
     75                  80                  85

CAT GAG AAT GAT GAA GGC CAC CAG GTA AAG TGG TCG GCT AGG GAA GGT        460
His Glu Asn Asp Glu Gly His Gln Val Lys Trp Ser Ala Arg Glu Gly
 90                  95                 100                 105

GTA GAC CTT GGC ATA TCC GGG CTC ACG ACG GTG TCT GGG CCT GAG TGG        508
Val Asp Leu Gly Ile Ser Gly Leu Thr Thr Val Ser Gly Pro Glu Trp
                110                 115                 120

AAT ATG TGC CCG CTA CCA CCA GTT GAC CAA AGG AGC ACG ACA CCT GCA        556
Asn Met Cys Pro Leu Pro Pro Val Asp Gln Arg Ser Thr Thr Pro Ala
            125                 130                 135

ACT GAG CCC ACA ATT GGT GAC ATG ATC GAA TTC TAT GAA GGG CAC ATC        604
Thr Glu Pro Thr Ile Gly Asp Met Ile Glu Phe Tyr Glu Gly His Ile
        140                 145                 150

TAT CAT TAT GCT ATA TAC ATA GGT CAA GGC AAG ACG GTG GGT GTA CAC        652
Tyr His Tyr Ala Ile Tyr Ile Gly Gln Gly Lys Thr Val Gly Val His
    155                 160                 165

TCC CCT CAA GCA GCC TTC TCA ATA ACG AGG ATC ACC ATA CAG CCC ATA        700
Ser Pro Gln Ala Ala Phe Ser Ile Thr Arg Ile Thr Ile Gln Pro Ile
170                 175                 180                 185

TCA GCT TGG TGG CGA GTC TGT TAT GTC CCA CAA CCA AAA CAG AGG CTC        748
Ser Ala Trp Trp Arg Val Cys Tyr Val Pro Gln Pro Lys Gln Arg Leu
                190                 195                 200

ACA TAC GAC CAA CTC AAA GAA TTA GAA AAT GAA CCA TGG CCG TAT GCC        796
Thr Tyr Asp Gln Leu Lys Glu Leu Glu Asn Glu Pro Trp Pro Tyr Ala
            205                 210                 215

GCA GTC ACG AAC AAC TGC TTC GAA TTT TGT TGC CAG GTC ATG TGC TTG        844
Ala Val Thr Asn Asn Cys Phe Glu Phe Cys Cys Gln Val Met Cys Leu
        220                 225                 230
```

Table 3, continued

```
GAA GAT ACT TGG TTG CAA AGG AAG CTC ATC TCC TCT GGC CGG TTT TAC         892
Glu Asp Thr Trp Leu Gln Arg Lys Leu Ile Ser Ser Gly Arg Phe Tyr
    235                 240                 245

CAC CCG ACC CAA GAT TGG TCC CGA GAC ACT CCA GAA TTC CAA CAA GAC         940
His Pro Thr Gln Asp Trp Ser Arg Asp Thr Pro Glu Phe Gln Gln Asp
250                 255                 260                 265

AGC AAG TTA GAG ATG GTT AGG GAT GCA GTG CTA GCC GCT ATA AAT GGG         988
Ser Lys Leu Glu Met Val Arg Asp Ala Val Leu Ala Ala Ile Asn Gly
                270                 275                 280

TTG GTG TCG CGG CCA TTT AAA GAT CTT CTG GGT AAG CTC AAA CCC TTG        1036
Leu Val Ser Arg Pro Phe Lys Asp Leu Leu Gly Lys Leu Lys Pro Leu
            285                 290                 295

AAC GTG CTT AAC TTA CTT TCA AAC TGT GAT TGG ACG TTC ATG GGG GTC        1084
Asn Val Leu Asn Leu Leu Ser Asn Cys Asp Trp Thr Phe Met Gly Val
        300                 305                 310

GTG GAG ATG GTG GTC CTC CTT TTA GAA CTC TTT GGA ATC TTT TGG AAC        1132
Val Glu Met Val Val Leu Leu Leu Glu Leu Phe Gly Ile Phe Trp Asn
    315                 320                 325

CCA CCT GAT GTT TCC AAC TTT ATA GCT TCA CTC CTG CCA GAT TTC CAT        1180
Pro Pro Asp Val Ser Asn Phe Ile Ala Ser Leu Leu Pro Asp Phe His
330                 335                 340                 345

CTA CAG GGC CCC GAG GAC CTT GCC AGG GAT CTC GTG CCA ATA GTA TTG        1228
Leu Gln Gly Pro Glu Asp Leu Ala Arg Asp Leu Val Pro Ile Val Leu
                350                 355                 360

GGG GGG ATC GGC TTA GCC ATA GGA TTC ACC AGA GAC AAG GTA AGT AAG        1276
Gly Gly Ile Gly Leu Ala Ile Gly Phe Thr Arg Asp Lys Val Ser Lys
            365                 370                 375

ATG ATG AAG AAT GCT GTT GAT GGA CTT CGT GCG GCA ACC CAG CTC GGT        1324
Met Met Lys Asn Ala Val Asp Gly Leu Arg Ala Ala Thr Gln Leu Gly
        380                 385                 390

CAA TAT GGC CTA GAA ATA TTC TCA TTA CTA AAG AAG TAC TTC TTC GGT        1372
Gln Tyr Gly Leu Glu Ile Phe Ser Leu Leu Lys Lys Tyr Phe Phe Gly
    395                 400                 405

GGT GAT CAA ACA GAG AAA ACC CTA AAA GAT ATT GAG TCA GCA GTT ATA        1420
Gly Asp Gln Thr Glu Lys Thr Leu Lys Asp Ile Glu Ser Ala Val Ile
410                 415                 420                 425

GAT ATG GAA GTA CTA TCA TCT ACA TCA GTG ACT CAG CTC GTG AGG GAC        1468
Asp Met Glu Val Leu Ser Ser Thr Ser Val Thr Gln Leu Val Arg Asp
                430                 435                 440

AAA CAG TCT GCA CGG GCT TAT ATG GCC ATC TTA GAT AAT GAA GAA GAA        1516
Lys Gln Ser Ala Arg Ala Tyr Met Ala Ile Leu Asp Asn Glu Glu Glu
            445                 450                 455

AAG GCA AGG AAA TTA TCT GTC AGG AAT GCC GAC CCA CAC GTA GTA TCC        1564
Lys Ala Arg Lys Leu Ser Val Arg Asn Ala Asp Pro His Val Val Ser
        460                 465                 470

TCT ACC AAT GCT CTC ATA TCC CGG ATC TCA ATG GCT AGG GCT GCA TTG        1612
Ser Thr Asn Ala Leu Ile Ser Arg Ile Ser Met Ala Arg Ala Ala Leu
    475                 480                 485
```

Table 3, continued

```
GCC AAG GCT CAA GCT GAA ATG ACC AGC AGG ATG CGT CCT GTG GTC ATT      1660
Ala Lys Ala Gln Ala Glu Met Thr Ser Arg Met Arg Pro Val Val Ile
490                 495                 500                 505

ATG ATG TGT GGG CCC CCT GGT ATA GGT AAA ACC AAG GCA GCA GAA CAT      1708
Met Met Cys Gly Pro Pro Gly Ile Gly Lys Thr Lys Ala Ala Glu His
                510                 515                 520

CTG GCT AAA CGC CTA GCC AAT GAG ATA CGG CCT GGT GGT AAG GTT GGG      1756
Leu Ala Lys Arg Leu Ala Asn Glu Ile Arg Pro Gly Gly Lys Val Gly
            525                 530                 535

CTG GTC CCA CGG GAG GCA GTG GAT CAT TGG GAT GGA TAT CAC GGA GAG      1804
Leu Val Pro Arg Glu Ala Val Asp His Trp Asp Gly Tyr His Gly Glu
        540                 545                 550

GAA GTG ATG CTG TGG GAC GAC TAT GGA ATG ACA AAG ATA CAG GAA GAC      1852
Glu Val Met Leu Trp Asp Asp Tyr Gly Met Thr Lys Ile Gln Glu Asp
    555                 560                 565

TGT AAT AAA CTG CAA GCC ATA GCC GAC TCA GCC CCC CTA ACA CTC AAT      1900
Cys Asn Lys Leu Gln Ala Ile Ala Asp Ser Ala Pro Leu Thr Leu Asn
570                 575                 580                 585

TGT GAC CGA ATA GAA AAC AAG GGA ATG CAA TTT GTG TCT GAT GCT ATA      1948
Cys Asp Arg Ile Glu Asn Lys Gly Met Gln Phe Val Ser Asp Ala Ile
                590                 595                 600

GTC ATC ACC ACC AAT GCT CCT GGC CCA GCC CCA GTG GAC TTT GTC AAC      1996
Val Ile Thr Thr Asn Ala Pro Gly Pro Ala Pro Val Asp Phe Val Asn
            605                 610                 615

CTC GGG CCT GTT TGC CGA AGG GTG GAC TTC CTT GTG TAT TGC ACG GCA      2044
Leu Gly Pro Val Cys Arg Arg Val Asp Phe Leu Val Tyr Cys Thr Ala
        620                 625                 630

CCT GAA GTT GAA CAC ACG AGG AAA GTC AGT CCT GGG GAC ACA ACT GCA      2092
Pro Glu Val Glu His Thr Arg Lys Val Ser Pro Gly Asp Thr Thr Ala
    635                 640                 645

CTG AAA GAC TGC TTC AAG CCC GAT TTC TCA CAT CTA AAA ATG GAG TTG      2140
Leu Lys Asp Cys Phe Lys Pro Asp Phe Ser His Leu Lys Met Glu Leu
650                 655                 660                 665

GCT CCC CAA GGG GGC TTT GAT AAC CAA GGG AAT ACC CCG TTT GGT AAG      2188
Ala Pro Gln Gly Gly Phe Asp Asn Gln Gly Asn Thr Pro Phe Gly Lys
                670                 675                 680

GGT GTG ATG AAG CCC ACC ACC ATA AAC AGG CTG TTA ATC CAG GCT GTA      2236
Gly Val Met Lys Pro Thr Thr Ile Asn Arg Leu Leu Ile Gln Ala Val
            685                 690                 695

GCC TTG ACG ATG GAG AGA CAG GAT GAG TTC CAA CTC CAG GGG CCT ACG      2284
Ala Leu Thr Met Glu Arg Gln Asp Glu Phe Gln Leu Gln Gly Pro Thr
        700                 705                 710

TAT GAC TTT GAT ACT GAC AGA GTA GCT GCG TTC ACG AGG ATG GCC CGA      2332
Tyr Asp Phe Asp Thr Asp Arg Val Ala Ala Phe Thr Arg Met Ala Arg
    715                 720                 725

GCC AAC GGG TTG GGT CTC ATA TCC ATG GCC TCC CTA GGC AAA AAG CTA      2380
Ala Asn Gly Leu Gly Leu Ile Ser Met Ala Ser Leu Gly Lys Lys Leu
730                 735                 740                 745
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Table 3, continued

```
CGC AGT GTC ACC ACT ATT GAA GGA TTA AAG AAT GCT CTA TCA GGC TAT        2428
Arg Ser Val Thr Thr Ile Glu Gly Leu Lys Asn Ala Leu Ser Gly Tyr
                750                 755                 760

AAA ATA TCA AAA TGC AGT ATA CAA TGG CAG TCA AGG GTG TAC ATT ATA        2476
Lys Ile Ser Lys Cys Ser Ile Gln Trp Gln Ser Arg Val Tyr Ile Ile
            765                 770                 775

GAA TCA GAT GGT GCC AGT GTA CAA ATC AAA GAA GAC AAG CAA GCT TTG        2524
Glu Ser Asp Gly Ala Ser Val Gln Ile Lys Glu Asp Lys Gln Ala Leu
        780                 785                 790

ACC CCT CTG CAG CAG ACA ATT AAC ACG GCC TCA CTT GCC ATC ACT CGA        2572
Thr Pro Leu Gln Gln Thr Ile Asn Thr Ala Ser Leu Ala Ile Thr Arg
    795                 800                 805

CTC AAA GCA GCT AGG GCT GTG GCA TAC GCT TCA TGT TTC CAG TCC GCC        2620
Leu Lys Ala Ala Arg Ala Val Ala Tyr Ala Ser Cys Phe Gln Ser Ala
810                 815                 820                 825

ATA ACT ACC ATA CTA CAA ATG GCG GGA TCT GCG CTC GTT ATT AAT CGA        2668
Ile Thr Thr Ile Leu Gln Met Ala Gly Ser Ala Leu Val Ile Asn Arg
                830                 835                 840

GCG GTC AAG CGT ATG TTT GGT ACC CGT ACA GCA GCC ATG GCA TTA GAA        2716
Ala Val Lys Arg Met Phe Gly Thr Arg Thr Ala Ala Met Ala Leu Glu
            845                 850                 855

GGA CCT GGG AAA GAA CAT AAT TGC AGG GTC CAT AAG GCT AAG GAA GCT        2764
Gly Pro Gly Lys Glu His Asn Cys Arg Val His Lys Ala Lys Glu Ala
        860                 865                 870

GGA AAG GGG CCC ATA GGT CAT GAT GAC ATG GTA GAA AGG TTT GGC CTA        2812
Gly Lys Gly Pro Ile Gly His Asp Asp Met Val Glu Arg Phe Gly Leu
    875                 880                 885

TGT GAA ACT GAA GAG GAG GAG AGT GAG GAC CAA ATT CAA ATG GTA CCA        2860
Cys Glu Thr Glu Glu Glu Glu Ser Glu Asp Gln Ile Gln Met Val Pro
890                 895                 900                 905

AGT GAT GCC GTC CCA GAA GGA AAG AAC AAA GGC AAG ACC AAA AAG GGA        2908
Ser Asp Ala Val Pro Glu Gly Lys Asn Lys Gly Lys Thr Lys Lys Gly
                910                 915                 920

CGT GGT CGC AAA AAT AAC TAT AAT GCA TTC TCT CGC CGT GGT CTG AGT        2956
Arg Gly Arg Lys Asn Asn Tyr Asn Ala Phe Ser Arg Arg Gly Leu Ser
            925                 930                 935

GAT GAA GAA TAT GAA GAG TAC AAA AAG ATC AGA GAA GAA AAG AAT GGC        3004
Asp Glu Glu Tyr Glu Glu Tyr Lys Lys Ile Arg Glu Glu Lys Asn Gly
        940                 945                 950

AAT TAT AGT ATA CAA GAA TAC TTG GAG GAC CGC CAA CGA TAT GAG GAA        3052
Asn Tyr Ser Ile Gln Glu Tyr Leu Glu Asp Arg Gln Arg Tyr Glu Glu
    955                 960                 965

GAA TTA GCA GAG GTA CAG GCA GGT GGT GAT GGT GGC ATA GGA GAA ACT        3100
Glu Leu Ala Glu Val Gln Ala Gly Gly Asp Gly Gly Ile Gly Glu Thr
970                 975                 980                 985

GAA ATG GAA ATC CGT CAC AGG GTC TTC TAT AAA TCC AAG AGT AAG AAA        3148
Glu Met Glu Ile Arg His Arg Val Phe Tyr Lys Ser Lys Ser Lys Lys
                990                 995                 1000
```

Table 3, continued

```
CAC CAA CAA GAG CAA CGG CGA CAA CTT GGT CTA GTG ACT GGA TCA GAC       3196
His Gln Gln Glu Gln Arg Arg Gln Leu Gly Leu Val Thr Gly Ser Asp
            1005                1010                1015

ATC AGA AAA CGT AAG CCC ATT GAC TGG ACC CCG CCA AAG AAT GAA TGG       3244
Ile Arg Lys Arg Lys Pro Ile Asp Trp Thr Pro Pro Lys Asn Glu Trp
        1020                1025                1030

GCA GAT GAT GAC AGA GAG GTG GAT TAT AAT GAA AAG ATC AAT TTT GAA       3292
Ala Asp Asp Asp Arg Glu Val Asp Tyr Asn Glu Lys Ile Asn Phe Glu
    1035                1040                1045

GCT CCC CCG ACA CTA TGG AGC CGA GTC ACA AAG TTT GGA TCA GGA TGG       3340
Ala Pro Pro Thr Leu Trp Ser Arg Val Thr Lys Phe Gly Ser Gly Trp
1050                1055                1060                1065

GGC TTT TGG GTC AGC CCG ACA GTG TTC ATC ACA ACC ACA CAT GTA GTG       3388
Gly Phe Trp Val Ser Pro Thr Val Phe Ile Thr Thr Thr His Val Val
                1070                1075                1080

CCA ACT GGT GTG AAA GAA TTC TTT GGT GAG CCC CTA TCT AGT ATA GCA       3436
Pro Thr Gly Val Lys Glu Phe Phe Gly Glu Pro Leu Ser Ser Ile Ala
            1085                1090                1095

ATC CAC CAA GCA GGT GAG TTC ACA CAA TTC AGG TTC TCA AAG AAA ATG       3484
Ile His Gln Ala Gly Glu Phe Thr Gln Phe Arg Phe Ser Lys Lys Met
        1100                1105                1110

CGC CCT GAC TTG ACA GGT ATG GTC CTT GAA GAA GGT TGC CCT GAA GGG       3532
Arg Pro Asp Leu Thr Gly Met Val Leu Glu Glu Gly Cys Pro Glu Gly
    1115                1120                1125

ACA GTC TGC TCA GTC CTA ATT AAA CGG GAT TCG GGT GAA CTA CTT CCG       3580
Thr Val Cys Ser Val Leu Ile Lys Arg Asp Ser Gly Glu Leu Leu Pro
1130                1135                1140                1145

CTA GCC GTC CGT ATG GGG GCT ATT GCC TCC ATG AGG ATA CAG GGT CGG       3628
Leu Ala Val Arg Met Gly Ala Ile Ala Ser Met Arg Ile Gln Gly Arg
                1150                1155                1160

CTT GTC CAT GGC CAA TCA GGG ATG TTA CTG ACA GGG GCC AAT GCA AAG       3676
Leu Val His Gly Gln Ser Gly Met Leu Leu Thr Gly Ala Asn Ala Lys
            1165                1170                1175

GGG ATG GAT CTT GGC ACT ATA CCA GGA GAC TGC GGG GCA CCA TAC GTC       3724
Gly Met Asp Leu Gly Thr Ile Pro Gly Asp Cys Gly Ala Pro Tyr Val
        1180                1185                1190

CAC AAG CGC GGG AAT GAC TGG GTT GTG TGT GGA GTC CAC GCT GCA GCC       3772
His Lys Arg Gly Asn Asp Trp Val Val Cys Gly Val His Ala Ala Ala
    1195                1200                1205

ACA AAG TCA GGC AAC ACC GTG GTC TGC GCT GTA CAG GCT GGA GAG GGC       3820
Thr Lys Ser Gly Asn Thr Val Val Cys Ala Val Gln Ala Gly Glu Gly
1210                1215                1220                1225

GAA ACC GCA CTA GAA GGT GGA GAC AAG GGG CAT TAT GCC GGC CAC GAG       3868
Glu Thr Ala Leu Glu Gly Gly Asp Lys Gly His Tyr Ala Gly His Glu
                1230                1235                1240

ATT GTG AGG TAT GGA AGT GGC CCA GCA CTG TCA ACT AAA ACA AAA TTC       3916
Ile Val Arg Tyr Gly Ser Gly Pro Ala Leu Ser Thr Lys Thr Lys Phe
            1245                1250                1255
```

July 25, 2002

Rebecca M. Hale
Chiron Corporation
P.O. Box 8097
Emeryville, CA 94662-8097

Re: U.S. Patent Application Serial No. 09/674,183 for "POLYEPITOPE CARRIER PROTEINS," by Rappuoli et al.
Your Reference: PP00362.102
Our Reference: 2302-0362

Dear Rebecca:

Enclosed are copies of the Sequence Listing and accompanying documents filed July 17, 2002 with the U.S. Patent & Trademark Office in the above-identified case.

We will keep you informed of further developments.

Very truly yours,

Roberta L. Robins

RLR/js
Enclosures

July 25, 2002

Rebecca M. Hale
Corporate Patent Counsel
Chiron Corporation
P.O. Box 8097
Emeryville, CA 94662-8097

Re: U.S. Patent Application Serial No. 09/674,183 for "POLYEPITOPE CARRIER PROTEINS," by Rappuoli et al.
Your Reference: PP00362.102
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Dear Rebecca:

Enclosed are copies of the Sequence Listing and accompanying documents filed July 17, 2002 with the U.S. Patent & Trademark Office in the above-identified case.

We will keep you informed of further developments.

Very truly yours,

Roberta L. Robins

RLR/js
Enclosures

July 25, 2002

Anne S. Dollard, Esq.
CHIRON CORPORATION
Intellectual Property - R440
P.O. Box 8097
Emeryville, CA 94662-8097

Re: U.S. Patent Application Serial No. 10/123,101 for "CHIMERIC ALPHAVIRUS REPLICON PARTICLES," by Polo et al.
Your Reference: PP17924.002
Our Reference: 2300-17924

Dear Anne:

Enclosed are copies of our Response to the Notice to File Missing Parts and accompanying documents which were filed with the U.S. Patent and Trademark Office on July 17, 2002 in the above-identified application.

We will keep you informed of further developments.

Very truly yours,

Dahna S. Pasternak

DSP/js
Enclosures

Table 3, continued

TGG AGG TCC TCC CCA GAA CCA CTG CCC CCC GGA GTA TAT GAG CCA GCA 3964
Trp Arg Ser Ser Pro Glu Pro Leu Pro Pro Gly Val Tyr Glu Pro Ala
1260 1265 1270

TAC CTG GGG GGC AAG GAC CCC CGT GTA CAG AAT GGC CCA TCC CTA CAA 4012
Tyr Leu Gly Gly Lys Asp Pro Arg Val Gln Asn Gly Pro Ser Leu Gln
1275 1280 1285

CAG GTA CTA CGT GAC CAA CTG AAA CCC TTT GCG GAC CCC CGC GGC CGC 4060
Gln Val Leu Arg Asp Gln Leu Lys Pro Phe Ala Asp Pro Arg Gly Arg
1290 1295 1300 1305

ATG CCT GAG CCT GGC CTA CTG GAG GCT GCG GTT GAG ACT GTA ACA TCC 4108
Met Pro Glu Pro Gly Leu Leu Glu Ala Ala Val Glu Thr Val Thr Ser
1310 1315 1320

ATG TTA GAA CAG ACA ATG GAT ACC CCA AGC CCG TGG TCT TAC GCT GAT 4156
Met Leu Glu Gln Thr Met Asp Thr Pro Ser Pro Trp Ser Tyr Ala Asp
1325 1330 1335

GCC TGC CAA TCT CTT GAC AAA ACT ACT AGT TCG GGG TAC CCT CAC CAT 4204
Ala Cys Gln Ser Leu Asp Lys Thr Thr Ser Ser Gly Tyr Pro His His
1340 1345 1350

AAA AGG AAG AAT GAT GAT TGG AAT GGC ACC ACC TTC GTT GGA GAG CTC 4252
Lys Arg Lys Asn Asp Asp Trp Asn Gly Thr Thr Phe Val Gly Glu Leu
1355 1360 1365

GGT GAG CAA GCT GCA CAC GCC AAC AAT ATG TAT GAG AAT GCT AAA CAT 4300
Gly Glu Gln Ala Ala His Ala Asn Asn Met Tyr Glu Asn Ala Lys His
1370 1375 1380 1385

ATG AAA CCC ATT TAC ACT GCA GCC TTA AAA GAT GAA CTA GTC AAG CCA 4348
Met Lys Pro Ile Tyr Thr Ala Ala Leu Lys Asp Glu Leu Val Lys Pro
1390 1395 1400

GAA AAG ATT TAT CAA AAA GTC AAG AAG CGT CTA CTA TGG GGC GCC GAT 4396
Glu Lys Ile Tyr Gln Lys Val Lys Lys Arg Leu Leu Trp Gly Ala Asp
1405 1410 1415

CTC GGA ACA GTG GTC AGG GCC GCC CGG GCT TTT GGC CCA TTT TGT GAC 4444
Leu Gly Thr Val Val Arg Ala Ala Arg Ala Phe Gly Pro Phe Cys Asp
1420 1425 1430

GCT ATA AAA TCA CAT GTC ATC AAA TTG CCA ATA AAA GTT GGC ATG AAC 4492
Ala Ile Lys Ser His Val Ile Lys Leu Pro Ile Lys Val Gly Met Asn
1435 1440 1445

ACA ATA GAA GAT GGC CCC CTC ATC TAT GCT GAG CAT GCT AAA TAT AAG 4540
Thr Ile Glu Asp Gly Pro Leu Ile Tyr Ala Glu His Ala Lys Tyr Lys
1450 1455 1460 1465

AAT CAT TTT GAT GCA GAT TAT ACA GCA TGG GAC TCA ACA CAA AAT AGA 4588
Asn His Phe Asp Ala Asp Tyr Thr Ala Trp Asp Ser Thr Gln Asn Arg
1470 1475 1480

CAA ATT ATG ACA GAA TCC TTC TCC ATT ATG TCG CGC CTT ACG GCC TCA 4636
Gln Ile Met Thr Glu Ser Phe Ser Ile Met Ser Arg Leu Thr Ala Ser
1485 1490 1495

CCA GAA TTG GCC GAG GTT GTG GCC CAA GAT TTG CTA GCA CCA TCT GAG 4684
Pro Glu Leu Ala Glu Val Val Ala Gln Asp Leu Leu Ala Pro Ser Glu
1500 1505 1510

Table 3, continued

```
ATG GAT GTA GGT GAT TAT GTC ATC AGG GTC AAA GAG GGG CTG CCA TCT        4732
Met Asp Val Gly Asp Tyr Val Ile Arg Val Lys Glu Gly Leu Pro Ser
    1515                1520                1525

GGA TTC CCA TGT ACT TCC CAG GTG AAC AGC ATA AAT CAC TGG ATA ATT        4780
Gly Phe Pro Cys Thr Ser Gln Val Asn Ser Ile Asn His Trp Ile Ile
1530                1535                1540                1545

ACT CTC TGT GCA CTG TCT GAG GCC ACT GGT TTA TCA CCT GAT GTG GTG        4828
Thr Leu Cys Ala Leu Ser Glu Ala Thr Gly Leu Ser Pro Asp Val Val
                1550                1555                1560

CAA TCC ATG TCA TAT TTC TCA TTT TAT GGT GAT GAT GAG ATT GTG TCA        4876
Gln Ser Met Ser Tyr Phe Ser Phe Tyr Gly Asp Asp Glu Ile Val Ser
            1565                1570                1575

ACT GAC ATA GAT TTT GAC CCA GCC CGC CTC ACT CAA ATT CTC AAG GAA        4924
Thr Asp Ile Asp Phe Asp Pro Ala Arg Leu Thr Gln Ile Leu Lys Glu
        1580                1585                1590

TAT GGC CTC AAA CCA ACA AGG CCT GAC AAA ACA GAA GGA CCA ATA CAA        4972
Tyr Gly Leu Lys Pro Thr Arg Pro Asp Lys Thr Glu Gly Pro Ile Gln
    1595                1600                1605

GTG AGG AAA AAT GTG GAT GGA CTG GTC TTC TTG CGG CGC ACC ATT TCC        5020
Val Arg Lys Asn Val Asp Gly Leu Val Phe Leu Arg Arg Thr Ile Ser
1610                1615                1620                1625

CGT GAT GCG GCA GGG TTC CAA GGC AGG TTA GAT AGG GCT TCG ATT GAA        5068
Arg Asp Ala Ala Gly Phe Gln Gly Arg Leu Asp Arg Ala Ser Ile Glu
                1630                1635                1640

CGC CAA ATC TTC TGG ACC CGC GGG CCC AAT CAT TCA GAT CCA TCA GAG        5116
Arg Gln Ile Phe Trp Thr Arg Gly Pro Asn His Ser Asp Pro Ser Glu
            1645                1650                1655

ACT CTA GTG CCA CAC ACT CAA AGA AAA ATA CAG TTG ATT TCA CTT CTA        5164
Thr Leu Val Pro His Thr Gln Arg Lys Ile Gln Leu Ile Ser Leu Leu
        1660                1665                1670

GGG GAA GCT TCA CTC CAT GGT GAG AAA TTT TAC AGA AAG ATT TCC AGC        5212
Gly Glu Ala Ser Leu His Gly Glu Lys Phe Tyr Arg Lys Ile Ser Ser
    1675                1680                1685

AAG GTC ATA CAT GAA ATC AAG ACT GGT GGA TTG GAA ATG TAT GTC CCA        5260
Lys Val Ile His Glu Ile Lys Thr Gly Gly Leu Glu Met Tyr Val Pro
1690                1695                1700                1705

GGA TGG CAG GCC ATG TTC CGC TGG ATG CGC TTC CAT GAC CTC GGA TTG        5308
Gly Trp Gln Ala Met Phe Arg Trp Met Arg Phe His Asp Leu Gly Leu
                1710                1715                1720

TGG ACA GGA GAT CGC GAT CTT CTG CCC GAA TTC GTA AAT GAT GAT GGC        5356
Trp Thr Gly Asp Arg Asp Leu Leu Pro Glu Phe Val Asn Asp Asp Gly
            1725                1730                1735

GTC TAAGGACGCT ACATCAAGCG TGGATGGCGC TAGTGGCGCT GGTCAGTTGG             5409
Val
```

Table 4. The amino acid sequence deduced from nucleotides 5346 through 6935 of the Norwalk virus genome shown in Table 2.

```
CGTAA ATG ATG ATG GCG TCT AAG GAC GCT ACA TCA AGC GTG GAT GGC          5387
      Met Met Met Ala Ser Lys Asp Ala Thr Ser Ser Val Asp Gly
       1               5                  10

GCT AGT GGC GCT GGT CAG TTG GTA CCG GAG GTT AAT GCT TCT GAC CCT        5435
Ala Ser Gly Ala Gly Gln Leu Val Pro Glu Val Asn Ala Ser Asp Pro
 15                  20                  25                  30

CTT GCA ATG GAT CCT GTA GCA GGT TCT TCG ACA GCA GTC GCG ACT GCT        5483
Leu Ala Met Asp Pro Val Ala Gly Ser Ser Thr Ala Val Ala Thr Ala
                 35                  40                  45

GGA CAA GTT AAT CCT ATT GAT CCC TGG ATA ATT AAT AAT TTT GTG CAA        5531
Gly Gln Val Asn Pro Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln
             50                  55                  60

GCC CCC CAA GGT GAA TTT ACT ATT TCC CCA AAT AAT ACC CCC GGT GAT        5579
Ala Pro Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp
         65                  70                  75

GTT TTG TTT GAT TTG AGT TTG GGT CCC CAT CTT AAT CCT TTC TTG CTC        5627
Val Leu Phe Asp Leu Ser Leu Gly Pro His Leu Asn Pro Phe Leu Leu
     80                  85                  90

CAT CTA TCA CAA ATG TAT AAT GGT TGG GTT GGT AAC ATG AGA GTC AGG        5675
His Leu Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg
 95                 100                 105                 110

ATT ATG CTA GCT GGT AAT GCC TTT ACT GCG GGG AAG ATA ATA GTT TCC        5723
Ile Met Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Ile Ile Val Ser
                115                 120                 125

TGC ATA CCC CCT GGT TTT GGT TCA CAT AAT CTT ACT ATA GCA CAA GCA        5771
Cys Ile Pro Pro Gly Phe Gly Ser His Asn Leu Thr Ile Ala Gln Ala
            130                 135                 140

ACT CTC TTT CCA CAT GTG ATT GCT GAT GTT AGG ACT CTA GAC CCC ATT        5819
Thr Leu Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Asp Pro Ile
        145                 150                 155

GAG GTG CCT TTG GAA GAT GTT AGG AAT GTT CTC TTT CAT AAT AAT GAT        5867
Glu Val Pro Leu Glu Asp Val Arg Asn Val Leu Phe His Asn Asn Asp
    160                 165                 170

AGA AAT CAA CAA ACC ATG CGC CTT GTG TGC ATG CTG TAC ACC CCC CTC        5915
Arg Asn Gln Gln Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu
175                 180                 185                 190

CGC ACT GGT GGT GGT ACT GGT GAT TCT TTT GTA GTT GCA GGG CGA GTT        5963
Arg Thr Gly Gly Gly Thr Gly Asp Ser Phe Val Val Ala Gly Arg Val
                195                 200                 205

ATG ACT TGC CCC AGT CCT GAT TTT AAT TTC TTG TTT TTA GTC CCT CCT        6011
Met Thr Cys Pro Ser Pro Asp Phe Asn Phe Leu Phe Leu Val Pro Pro
            210                 215                 220

ACG GTG GAG CAG AAA ACC AGG CCC TTC ACA CTC CCA AAT CTG CCA TTG        6059
Thr Val Glu Gln Lys Thr Arg Pro Phe Thr Leu Pro Asn Leu Pro Leu
        225                 230                 235
```

Table 4, continued

```
AGT TCT CTG TCT AAC TCA CGT GCC CCT CTC CCA ATC AGT AGT ATG GGC      6107
Ser Ser Leu Ser Asn Ser Arg Ala Pro Leu Pro Ile Ser Ser Met Gly
    240                 245                 250

ATT TCC CCA GAC AAT GTC CAG AGT GTG CAG TTC CAA AAT GGT CGG TGT      6155
Ile Ser Pro Asp Asn Val Gln Ser Val Gln Phe Gln Asn Gly Arg Cys
255                 260                 265                 270

ACT CTG GAT GGC CGC CTG GTT GGC ACC ACC CCA GTT TCA TTG TCA CAT      6203
Thr Leu Asp Gly Arg Leu Val Gly Thr Thr Pro Val Ser Leu Ser His
                275                 280                 285

GTT GCC AAG ATA AGA GGG ACC TCC AAT GGC ACT GTA ATC AAC CTT ACT      6251
Val Ala Lys Ile Arg Gly Thr Ser Asn Gly Thr Val Ile Asn Leu Thr
            290                 295                 300

GAA TTG GAT GGC ACA CCC TTT CAC CCT TTT GAG GGC CCT GCC CCC ATT      6299
Glu Leu Asp Gly Thr Pro Phe His Pro Phe Glu Gly Pro Ala Pro Ile
        305                 310                 315

GGG TTT CCA GAC CTC GGT GGT TGT GAT TGG CAT ATC AAT ATG ACA CAG      6347
Gly Phe Pro Asp Leu Gly Gly Cys Asp Trp His Ile Asn Met Thr Gln
    320                 325                 330

TTT GGC CAT TCT AGC CAG ACC CAG TAT GAT GTA GAC ACC ACC CCT GAC      6395
Phe Gly His Ser Ser Gln Thr Gln Tyr Asp Val Asp Thr Thr Pro Asp
335                 340                 345                 350

ACT TTT GTC CCC CAT CTT GGT TCA ATT CAG GCA AAT GGC ATT GGC AGT      6443
Thr Phe Val Pro His Leu Gly Ser Ile Gln Ala Asn Gly Ile Gly Ser
                355                 360                 365

GGT AAT TAT GTT GGT GTT CTT AGC TGG ATT TCC CCC CCA TCA CAC CCG      6491
Gly Asn Tyr Val Gly Val Leu Ser Trp Ile Ser Pro Pro Ser His Pro
            370                 375                 380

TCT GGC TCC CAA GTT GAC CTT TGG AAG ATC CCC AAT TAT GGG TCA AGT      6539
Ser Gly Ser Gln Val Asp Leu Trp Lys Ile Pro Asn Tyr Gly Ser Ser
        385                 390                 395

ATT ACG GAG GCA ACA CAT CTA GCC CCT TCT GTA TAC CCC CCT GGT TTC      6587
Ile Thr Glu Ala Thr His Leu Ala Pro Ser Val Tyr Pro Pro Gly Phe
    400                 405                 410

GGA GAG GTA TTG GTC TTT TTC ATG TCA AAA ATG CCA GGT CCT GGT GCT      6635
Gly Glu Val Leu Val Phe Phe Met Ser Lys Met Pro Gly Pro Gly Ala
415                 420                 425                 430

TAT AAT TTG CCC TGT CTA TTA CCA CAA GAG TAC ATT TCA CAT CTT GCT      6683
Tyr Asn Leu Pro Cys Leu Leu Pro Gln Glu Tyr Ile Ser His Leu Ala
                435                 440                 445

AGT GAA CAA GCC CCT ACT GTA GGT GAG GCT GCC CTG CTC CAC TAT GTT      6731
Ser Glu Gln Ala Pro Thr Val Gly Glu Ala Ala Leu Leu His Tyr Val
            450                 455                 460

GAC CCT GAT ACC GGT CGG AAT CTT GGG GAA TTC AAA GCA TAC CCT GAT      6779
Asp Pro Asp Thr Gly Arg Asn Leu Gly Glu Phe Lys Ala Tyr Pro Asp
        465                 470                 475

GGT TTC CTC ACT TGT GTC CCC AAT GGG GCT AGC TCG GGT CCA CAA CAG      6827
Gly Phe Leu Thr Cys Val Pro Asn Gly Ala Ser Ser Gly Pro Gln Gln
    480                 485                 490
```

Table 4, continued

```
CTG CCG ATC AAT GGG GTC TTT GTC TTT GTT TCA TGG GTG TCC AGA TTT        6875
Leu Pro Ile Asn Gly Val Phe Val Phe Val Ser Trp Val Ser Arg Phe
495                 500                 505                 510

TAT CAA TTA AAG CCT GTG GGA ACT GCC AGC TCG GCA AGA GGT AGG CTT        6923
Tyr Gln Leu Lys Pro Val Gly Thr Ala Ser Ser Ala Arg Gly Arg Leu
                515                 520                 525

GGT CTG CGC CGA TAATGGCCCA AGCCATAATT GGTGCAATTG CTGCTTCCAC            6975
Gly Leu Arg Arg
            530
```

Table 5. The amino acid sequence deduced from nucleotides 6938 through 7573 of the Norwalk virus genome shown in Table 2.

```
CCAGCTCGGC AAGAGGTAGG CTTGGTCTGC GCCGATA ATG GCC CAA GCC ATA ATT      6955
                                         Met Ala Gln Ala Ile Ile
                                          1               5

GGT GCA ATT GCT GCT TCC ACA GCA GGT AGT GCT CTG GGA GCG GGC ATA      7003
Gly Ala Ile Ala Ala Ser Thr Ala Gly Ser Ala Leu Gly Ala Gly Ile
            10                  15                  20

CAG GTT GGT GGC GAA GCG GCC CTC CAA AGC CAA AGG TAT CAA CAA AAT      7051
Gln Val Gly Gly Glu Ala Ala Leu Gln Ser Gln Arg Tyr Gln Gln Asn
        25                  30                  35

TTG CAA CTG CAA GAA AAT TCT TTT AAA CAT GAC AGG GAA ATG ATT GGG      7099
Leu Gln Leu Gln Glu Asn Ser Phe Lys His Asp Arg Glu Met Ile Gly
    40                  45                  50

TAT CAG GTT GAA GCT TCA AAT CAA TTA TTG GCT AAA AAT TTG GCA ACT      7147
Tyr Gln Val Glu Ala Ser Asn Gln Leu Leu Ala Lys Asn Leu Ala Thr
 55                  60                  65                  70

AGA TAT TCA CTC CTC CGT GCT GGG GGT TTG ACC AGT GCT GAT GCA GCA      7195
Arg Tyr Ser Leu Leu Arg Ala Gly Gly Leu Thr Ser Ala Asp Ala Ala
                75                  80                  85

AGA TCT GTG GCA GGA GCT CCA GTC ACC CGC ATT GTA GAT TGG AAT GGC      7243
Arg Ser Val Ala Gly Ala Pro Val Thr Arg Ile Val Asp Trp Asn Gly
            90                  95                 100

GTG AGA GTG TCT GCT CCC GAG TCC TCT GCT ACC ACA TTG AGA TCC GGT      7291
Val Arg Val Ser Ala Pro Glu Ser Ser Ala Thr Thr Leu Arg Ser Gly
        105                 110                 115

GGC TTC ATG TCA GTT CCC ATA CCA TTT GCC TCT AAG CAA AAA CAG GTT      7339
Gly Phe Met Ser Val Pro Ile Pro Phe Ala Ser Lys Gln Lys Gln Val
    120                 125                 130

CAA TCA TCT GGT ATT AGT AAT CCA AAT TAT TCC CCT TCA TCC ATT TCT      7387
Gln Ser Ser Gly Ile Ser Asn Pro Asn Tyr Ser Pro Ser Ser Ile Ser
135                 140                 145                 150

CGA ACC ACT AGT TGG GTC GAG TCA CAA AAC TCA TCG AGA TTT GGA AAT      7435
Arg Thr Thr Ser Trp Val Glu Ser Gln Asn Ser Ser Arg Phe Gly Asn
                155                 160                 165

CTT TCT CCA TAC CAC GCG GAG GCT CTC AAT ACA GTG TGG TTG ACT CCA      7483
Leu Ser Pro Tyr His Ala Glu Ala Leu Asn Thr Val Trp Leu Thr Pro
            170                 175                 180

CCC GGT TCA ACA GCC TCT TCT ACA CTG TCT TCT GTG CCA CGT GGT TAT      7531
Pro Gly Ser Thr Ala Ser Ser Thr Leu Ser Ser Val Pro Arg Gly Tyr
        185                 190                 195

TTC AAT ACA GAC AGG TTG CCA TTA TTC GCA AAT AAT AGG CGA              7573
Phe Asn Thr Asp Arg Leu Pro Leu Phe Ala Asn Asn Arg Arg
    200                 205                 210
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Table 6. Primers used for detection of Norwalk-related virus by PCR

P-35	4944	5′ CTT GTT GGT TTG AGG CCA TAT	4924
P-36	4475	5′ ATA AAA GTT GGC ATG AAC A	4493
P-39	4878	5′ GTT GAC ACA ATC TCA TCA TC	4859
P-69	4721	5′ GGC CTG CCA TCT GGA TTG CC	4740
P-78	1670	5′ GGG CCC CCT GGT ATA GGT AA	1689
P-80	1931	5′ TGG TGA TGA CTA TAG CAT CAG ACA CAA A	1958
P-56*	4903	5′ ACT CAC CCA AAT CCT CCA	4920
P-23	5230	5′ GTT CTG ACC ACC TAA CCT	5247
P-42	5595	5′ AGT TTG GGT CCC CAT CTT AAT CCT TT	5620
P-55	5730	5′ TGA ACC AAA ACC AGG GGG	5747
P-58*	5210	5′ AGC AAA GTC ATA CAT GAA AT	5229
P-59	5634	5′ CCA TTA TAC ATT TGT AG	5650
P-60*	5712	5′ ATT ATA GTT TCT TGC ATA	5729
P-61	6115	5′ CAC ACT CTG GAC ATT GTC TG	6134
P-72	6296	5′ CAT TGG GTT TCC AGA CCT A	6313
P-63	6511	5′ ATA ATT GGG GAT CTT CCA AA	6530
P-76*	6095	5′ TAG TGG CAT GGG TAT TTC	6114
P-77	6316	5′ TAT GCC AAT CAC AGC CAC	6333
P-64	6491	5′ GTC TGG CTC CCA AGT TGA CC	6510
P-75	6726	5′ CGG TAT CAG GGT CAA CAT	6744
P-74	6707	5′ TGA GGC TGC CCT GCT CCA	6724
P-3	7009	5′ CCA CCG CTG TCC GGG AGG	7027
P-36 (New)#	4475	5′ GTT GCT GTT GGC ATT AAC A	4493

*Based on KY89 sequence # Based HuVc Sapporo sequence

Table 7. Detection of Norwalk and Norwalk-Related Viruses

Virus	NV ELISA	Polymerase PCR (36-35)	Polymerase PCR (69-39)	2C PCR (78-80)	Size of cDNA (bp)	YGDD motif	% na similarity to NV	% aa similarity to NV	Virus or Reference
8FIIa NV/68	+	+	+	+	470	yes	100	100	A. Kapikian
SRSV-3/88	+	+	ND	ND	470	yes	87	99	T. Ando
SRSV/KY/89	+	+	+	+	470	yes	87	99	I. Oishi
SRSV/CDC6/91	+	-	+	ND	118•	N/A	80	89	C. Moe/R. Glass
Desert Storm/90	-	+*	ND	ND	406*	yes	73	85	K. Green/J. Lew
SRSV/UT/88	-	+	+	ND	118•	N/A	71	82	P. Johnson
SMA/79	-	+	ND	ND	464	yes	63	60	P. Madore
SRSV/Cambridge, UK/92	-	+	+	-	469	yes	63	60	U. Desselberger
Toronto/91	ND	+*	ND	ND	470	yes	63	60	K. Green/M. Petric
CDC 32	-	-	+	ND	117•	N/A	62	66	C. Moe/R. Glass
HuCV Sapporo/82	-	+	ND	ND	488	yes	26	24	S. Nakata/D. Matson
Primate CV	ND	+	ND	ND	~470	yes	20	ND#	A. Smith/D. Matson
HuCV Houston DCC	ND	+	ND	ND	485	yes	17	12	D. Matson
HuCV Houston Child	ND	+	ND	ND	474	yes	9.8	6	D. Matson
Astro/CDC 37	-	+	+	ND	400	N/A	0	0	C. Moe/R. Glass

ND = Not done. N/A = Not available

* Internal primers were used to amplify this agent.

\# The primate CV 35-36 PCR product sequence is not yet complete. Similarity information is based upon the partial sequence.

• Size of 69-39 PCR cDNA product. Primers 69-39 are located inside 36-35 on the Norwalk virus genome sequence. All others are the sizes of the cDNA products made using primers 36-35.

Table 8. Nucleotide Homologies of Different Caliciviruses in the Primer 36 Region.

Virus Strain	Nucleotide*																							
Norwalk Virus	A	T	A	A	A	A	G	T	T	G	G	C	A	T	G	A	A	C	A	C	A	A	A	T
HuCV Sapporo	G	-	T	G	C	T	-	-	-	-	-	-	-	-	T	-	-	-	-	T	G	G	-	C
Feline CV F4	-	T	T	C	C	T	-	-	-	-	-	-	-	-	A	-	-	-	-	T	G	G	-	C
Feline CV CFI	-	-	-	C	C	G	-	-	-	-	-	-	-	-	T	-	-	-	-	T	G	G	-	C
Feline CV F9	-	-	-	C	-	G	-	-	-	-	-	T	-	-	C	-	-	-	-	T	G	G	-	C
Rabbit CV	-	-	T	G	C	-	-	-	-	-	-	-	G	-	T	G	-	-	-	T	G	-	C	A

* "-" means nucleotide is identical at that site for the strain. A new letter at a site indicates the nucleotide differs at that site. Primer 36 extends across the first 19 nucleotides in the Norwalk sequence above. Primer "new 36" is the first 19 nucleotides of the HuCV Sapporo sequence.

Table 9. Characterization of Serum from Animals Immunized Parenterally with Recombinant Norwalk Virus Particles[a]

	ELISA Titer of Indicated Serum with Norwalk Virus Particles	
Species Immunized	Pre-immune	Post-immune[c]
6 mice	<100[b]	>10^6
4 guinea pigs	<100	>10^6
2 rabbits	<100	>10^6

a Two to six animals of each species were immunized with purified recombinant Norwalk virus protein produced using the baculovirus expression system. Serum was collected before or after three immunizations with antigen (80mg for mice, 200mg for guinea pigs and 300mg for rabbits) and tested for reactivity with antigen coated on ELISA plates.

b Lowest dilution tested was 1:100.

c These sera also were used as capture and detector antibodies to establish an ELISA to detect Norwalk virus antigen.

Table 10. Nucleotide and amino acid sequence of human calicivirus Houston cDNA:

```
                                                                 44
GG CCA TGT TAT AGT GGT GTT CAC ATG AAA GAT GGC GAC AAG ATG         HuCV Houston
    P   C   Y   S   G   V   H   M   K   D   G   D   K   M          HuCV Houston amino acid

                                                                 89
TTG ATA GAT GCC AAT CTT CCT TAC AAC CAG AAA TTA ACT ACT ATG        HuCV Houston
 L   I   D   A   N   L   P   Y   N   Q   K   L   T   T   M         HuCV Houston amino acid

                                                                 134
ATT CAT GAG ACT AGG CAT AGG ATA GGA CAG TAT ATA GAT AAT ACT        HuCV Houston
 I   H   E   T   R   H   R   I   G   Q   Y   I   D   N   T         HuCV Houston amino acid

                                                                 179
TTT GGA AAG ACA TTT AGA CAT GGA TTG ACA AAA CCT GCT GAC AAG        HuCV Houston
 F   G   K   T   F   R   H   G   L   T   K   P   A   D   K         HuCV Houston amino acid

                                                                 224
ACT GTA GAT TTG ATC TAT AAG ACA TTG AAT TAT GAT GAT TTT CTG        HuCV Houston
 T   V   D   L   I   Y   K   T   L   N   Y   D   D   F   L         HuCV Houston amino acid

                                                                 269
GCA ATA ATG CTA ATC ATA TAT GGG CAA AAG TCG GCC ACT AAT ACG        HuCV Houston
 A   I   M   L   I   I   Y   G   Q   K   S   A   T   N   T         HuCV Houston amino acid

                                                                 314
GAG TTG CAA TTC TTG ATG GAG AAA CTT AGA GGT TAT GAA TCT ACA        HuCV Houston
 E   L   Q   F   L   M   E   K   L   R   G   Y   E   S   T         HuCV Houston amino acid

                                                                 359
ATG GAT GAC ATA GGG AAA GTC TAT GGA GAT GAT AAA ATG AGA GAT        HuCV Houston
 M   D   D   I   G   K   V   Y   G   D   D   K   M   R   D         HuCV Houston amino acid

                                                                 404
ATA ATC AAG AAT ATT TCT GAT GAT GAC ATA AAG AGT CTT TTA GGG        HuCV Houston
 I   I   K   N   I   S   D   D   D   I   K   S   L   L   G         HuCV Houston amino acid

                                                                 434
GAG ATA AAT AGT GAT TAT TCT GGT AAG NAT                            HuCV Houston
 E   I   N   S   D   Y   S   G   K   X                             Houston amino acid
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Matson, David O
Estes, Mary K
Jiang, Xi
Graham, David Y

(ii) TITLE OF INVENTION: Methods and Reagents to Detect and Characterize Norwalk and Related Viruses

(iii) NUMBER OF SEQUENCES: 75

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 1301 McKinney, Suite 5100
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(D) STATE: Texas
(E) COUNTRY: USA
(F) ZIP: 77010-3095

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 33,035
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(B) TELEFAX: 713-651-5246
(C) TELEX: Western Union 762829

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Norwalk virus
(B) STRAIN: 8FIIa
(C) INDIVIDUAL ISOLATE: 8FIIa

(vii) IMMEDIATE SOURCE:
(B) CLONE: pUCNV-953 and its derivatives

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 146..5359
(D) OTHER INFORMATION: /note= "The protein encoded by nucleotides 146 through 5359 is eventually cleaved to make at least a picornavirus 2c-like protein, a 3C-like protease and an RNA-dependent RNA plymerase.

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 5346..6935
(D) OTHER INFORMATION: /note= "Nucleotides 5346 through 5359 are used for coding two different amino acid sequences: the first is the amino acid is coded by nucleotide 146 through 5359, the second by nucleotides 5346 through 6935.

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 6938..7573

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGCGTCAAAA GACGTCGTTC CTACTGCTGC TAGCAGTGAA AATGCTAACA ACAATAGTAG     60
TATTAAGTCT CGTCTATTGG CGAGACTCAA GGGTTCAGGT GGGGCTACGT CCCCACCCAA    120
CTCGATAAAG ATAACCAACC AAGATATGGC TCTGGGGCTG ATTGGACAGG TCCCAGCGCC    180
AAAGGCCACA TCCGTCGATG TCCCTAAACA ACAGAGGGAT AGACCACCAC GGACTGTTGC    240
CGAAGTTCAA CAAAATTTGC GTTGGACTGA GAGACCACAA GACCAGAATG TTAAGACGTG    300
GGATGAGCTT GACCACACAA CAAAACAACA GATACTTGAT GAACACGCTG AGTGGTTTGA    360
TGCCGGTGGC TTAGGTCCAA GTACACTACC CACTAGTCAT GAACGGTACA CACATGAGAA    420
TGATGAAGGC CACCAGGTAA AGTGGTCGGC TAGGGAAGGT GTAGACCTTG GCATATCCGG    480
GCTCACGACG GTGTCTGGGC CTGAGTGGAA TATGTGCCCG CTACCACCAG TTGACCAAAG    540
GAGCACGACA CCTGCAACTG AGCCCACAAT TGGTGACATG ATCGAATTCT ATGAAGGGCA    600
CATCTATCAT TATGCTATAT ACATAGGTCA AGGCAAGACG GTGGGTGTAC ACTCCCCTCA    660
AGCAGCCTTC TCAATAACGA GGATCACCAT ACAGCCCATA TCAGCTTGGT GGCGAGTCTG    720
TTATGTCCCA CAACCAAAAC AGAGGCTCAC ATACGACCAA CTCAAAGAAT TAGAAAATGA    780
ACCATGGCCG TATGCCGCAG TCACGAACAA CTGCTTCGAA TTTTGTTGCC AGGTCATGTG    840
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CCAAGATTGG TCCCGAGACA CTCCAGAATT CCAACAAGAC AGCAAGTTAG AGATGGTTAG    960
GGATGCAGTG CTAGCCGCTA TAAATGGGTT GGTGTCGCGG CCATTTAAAG ATCTTCTGGG   1020
TAAGCTCAAA CCCTTGAACG TGCTTAACTT ACTTTCAAAC TGTGATTGGA CGTTCATGGG   1080
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TACATCAGTG ACTCAGCTCG TGAGGGACAA ACAGTCTGCA CGGGCTTATA TGGCCATCTT   1500
AGATAATGAA GAAGAAAAGG CAAGGAAATT ATCTGTCAGG AATGCCGACC CACACGTAGT   1560
ATCCTCTACC AATGCTCTCA TATCCCGGAT CTCAATGGCT AGGGCTGCAT TGGCCAAGGC   1620
TCAAGCTGAA ATGACCAGCA GGATGCGTCC TGTGGTCATT ATGATGTGTG GGCCCCCTGG   1680
TATAGGTAAA ACCAAGGCAG CAGAACATCT GGCTAAACGC CTAGCCAATG AGATACGGCC   1740
TGGTGGTAAG GTTGGGCTGG TCCCACGGGA GGCAGTGGAT CATTGGGATG GATATCACGG   1800
AGAGGAAGTG ATGCTGTGGG ACGACTATGG AATGACAAAG ATACAGGAAG ACTGTAATAA   1860
ACTGCAAGCC ATAGCCGACT CAGCCCCCCT AACACTCAAT TGTGACCGAA TAGAAAACAA   1920
GGGAATGCAA TTTGTGTCTG ATGCTATAGT CATCACCACC AATGCTCCTG GCCCAGCCCC   1980
AGTGGACTTT GTCAACCTCG GGCCTGTTTG CCGAAGGGTG GACTTCCTTG TGTATTGCAC   2040
GGCACCTGAA GTTGAACACA CGAGGAAAGT CAGTCCTGGG GACACAACTG CACTGAAAGA   2100
CTGCTTCAAG CCCGATTTCT CACATCTAAA AATGGAGTTG GCTCCCCAAG GGGGCTTTGA   2160
TAACCAAGGG AATACCCCGT TTGGTAAGGG TGTGATGAAG CCCACCACCA TAAACAGGCT   2220
GTTAATCCAG GCTGTAGCCT TGACGATGGA GAGACAGGAT GAGTTCCAAC TCCAGGGGCC   2280
TACGTATGAC TTTGATACTG ACAGAGTAGC TGCGTTCACG AGGATGGCCC GAGCCAACGG   2340
GTTGGGTCTC ATATCCATGG CCTCCCTAGG CAAAAAGCTA CGCAGTGTCA CCACTATTGA   2400
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AAGGGTGTAC ATTATAGAAT CAGATGGTGC CAGTGTACAA ATCAAAGAAG ACAAGCAAGC   2520
TTTGACCCCT CTGCAGCAGA CAATTAACAC GGCCTCACTT GCCATCACTC GACTCAAAGC   2580
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AGCTAGGGCT	GTGGCATACG	CTTCATGTTT	CCAGTCCGCC	ATAACTACCA	TACTACAAAT	2640
GGCGGGATCT	GCGCTCGTTA	TTAATCGAGC	GGTCAAGCGT	ATGTTTGGTA	CCCGTACAGC	2700
AGCCATGGCA	TTAGAAGGAC	CTGGGAAAGA	ACATAATTGC	AGGGTCCATA	AGGCTAAGGA	2760
AGCTGGAAAG	GGGCCCATAG	GTCATGATGA	CATGGTAGAA	AGGTTTGGCC	TATGTGAAAC	2820
TGAAGAGGAG	GAGAGTGAGG	ACCAAATTCA	AATGGTACCA	AGTGATGCCG	TCCCAGAAGG	2880
AAAGAACAAA	GGCAAGACCA	AAAAGGGACG	TGGTCGCAAA	AATAACTATA	ATGCATTCTC	2940
TCGCCGTGGT	CTGAGTGATG	AAGAATATGA	AGAGTACAAA	AAGATCAGAG	AAGAAAAGAA	3000
TGGCAATTAT	AGTATACAAG	AATACTTGGA	GGACCGCCAA	CGATATGAGG	AAGAATTAGC	3060
AGAGGTACAG	GCAGGTGGTG	ATGGTGGCAT	AGGAGAAACT	GAAATGGAAA	TCCGTCACAG	3120
GGTCTTCTAT	AAATCCAAGA	GTAAGAAACA	CCAACAAGAG	CAACGGCGAC	AACTTGGTCT	3180
AGTGACTGGA	TCAGACATCA	GAAAACGTAA	GCCCATTGAC	TGGACCCCGC	CAAAGAATGA	3240
ATGGGCAGAT	GATGACAGAG	AGGTGGATTA	TAATGAAAAG	ATCAATTTTG	AAGCTCCCCC	3300
GACACTATGG	AGCCGAGTCA	CAAAGTTTGG	ATCAGGATGG	GGCTTTTGGG	TCAGCCCGAC	3360
AGTGTTCATC	ACAACCACAC	ATGTAGTGCC	AACTGGTGTG	AAAGAATTCT	TTGGTGAGCC	3420
CCTATCTAGT	ATAGCAATCC	ACCAAGCAGG	TGAGTTCACA	CAATTCAGGT	TCTCAAAGAA	3480
AATGCGCCCT	GACTTGACAG	GTATGGTCCT	TGAAGAAGGT	TGCCCTGAAG	GGACAGTCTG	3540
CTCAGTCCTA	ATTAAACGGG	ATTCGGGTGA	ACTACTTCCG	CTAGCCGTCC	GTATGGGGGC	3600
TATTGCCTCC	ATGAGGATAC	AGGGTCGGCT	TGTCCATGGC	CAATCAGGGA	TGTTACTGAC	3660
AGGGGCCAAT	GCAAAGGGGA	TGGATCTTGG	CACTATACCA	GGAGACTGCG	GGCACCATA	3720
CGTCCACAAG	CGCGGGAATG	ACTGGGTTGT	GTGTGGAGTC	CACGCTGCAG	CCACAAAGTC	3780
AGGCAACACC	GTGGTCTGCG	CTGTACAGGC	TGGAGAGGGC	GAAACCGCAC	TAGAAGGTGG	3840
AGACAAGGGG	CATTATGCCG	GCCACGAGAT	TGTGAGGTAT	GGAAGTGGCC	CAGCACTGTC	3900
AACTAAAACA	AAATTCTGGA	GGTCCTCCCC	AGAACCACTG	CCCCCCGGAG	TATATGAGCC	3960
AGCATACCTG	GGGGGCAAGG	ACCCCCGTGT	ACAGAATGGC	CCATCCCTAC	AACAGGTACT	4020
ACGTGACCAA	CTGAAACCCT	TTGCGGACCC	CCGCGGCCGC	ATGCCTGAGC	CTGGCCTACT	4080
GGAGGCTGCG	GTTGAGACTG	TAACATCCAT	GTTAGAACAG	ACAATGGATA	CCCCAAGCCC	4140
GTGGTCTTAC	GCTGATGCCT	GCCAATCTCT	TGACAAAACT	ACTAGTTCGG	GGTACCCTCA	4200
CCATAAAAGG	AAGAATGATG	ATTGGAATGG	CACCACCTTC	GTTGGAGAGC	TCGGTGAGCA	4260
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AGCCTTAAAA	GATGAACTAG	TCAAGCCAGA	AAAGATTTAT	CAAAAAGTCA	AGAAGCGTCT	4380
ACTATGGGGC	GCCGATCTCG	GAACAGTGGT	CAGGGCCGCC	CGGGCTTTTG	GCCCATTTTG	4440
TGACGCTATA	AAATCACATG	TCATCAAATT	GCCAATAAAA	GTTGGCATGA	ACACAATAGA	4500
AGATGGCCCC	CTCATCTATG	CTGAGCATGC	TAAATATAAG	AATCATTTTG	ATGCAGATTA	4560
TACAGCATGG	GACTCAACAC	AAAATAGACA	AATTATGACA	GAATCCTTCT	CCATTATGTC	4620
GCGCCTTACG	GCCTCACCAG	AATTGGCCGA	GGTTGTGGCC	CAAGATTTGC	TAGCACCATC	4680
TGAGATGGAT	GTAGGTGATT	ATGTCATCAG	GGTCAAAGAG	GGGCTGCCAT	CTGGATTCCC	4740
ATGTACTTCC	CAGGTGAACA	GCATAAATCA	CTGGATAATT	ACTCTCTGTG	CACTGTCTGA	4800
GGCCACTGGT	TTATCACCTG	ATGTGGTGCA	ATCCATGTCA	TATTTCTCAT	TTTATGGTGA	4860
TGATGAGATT	GTGTCAACTG	ACATAGATTT	TGACCCAGCC	CGCCTCACTC	AAATTCTCAA	4920
GGAATATGGC	CTCAAACCAA	CAAGGCCTGA	CAAAACAGAA	GGACCAATAC	AAGTGAGGAA	4980
AAATGTGGAT	GGACTGGTCT	TCTTGCGGCG	CACCATTTCC	CGTGATGCGG	CAGGGTTCCA	5040
AGGCAGGTTA	GATAGGGCTT	CGATTGAACG	CCAAATCTTC	TGGACCCGCG	GGCCCAATCA	5100
TTCAGATCCA	TCAGAGACTC	TAGTGCCACA	CACTCAAAGA	AAAATACAGT	TGATTTCACT	5160
TCTAGGGGAA	GCTTCACTCC	ATGGTGAGAA	ATTTTACAGA	AAGATTTCCA	GCAAGGTCAT	5220
ACATGAAATC	AAGACTGGTG	GATTGGAAAT	GTATGTCCCA	GGATGGCAGG	CCATGTTCCG	5280
CTGGATGCGC	TTCCATGACC	TCGGATTGTG	GACAGGAGAT	CGCGATCTTC	TGCCCGAATT	5340
CGTAAATGAT	GATGGCGTCT	AAGGACGCTA	CATCAAGCGT	GGATGGCGCT	AGTGGCGCTG	5400
GTCAGTTGGT	ACCGGAGGTT	AATGCTTCTG	ACCCTCTTGC	AATGGATCCT	GTAGCAGGTT	5460
CTTCGACAGC	AGTCGCGACT	GCTGGACAAG	TTAATCCTAT	TGATCCCTGG	ATAATTAATA	5520
ATTTTGTGCA	AGCCCCCCAA	GGTGAATTTA	CTATTTCCCC	AAATAATACC	CCCGGTGATG	5580
TTTTGTTTGA	TTTGAGTTTG	GGTCCCCATC	TTAATCCTTT	CTTGCTCCAT	CTATCACAAA	5640
TGTATAATGG	TTGGGTTGGT	AACATGAGAG	TCAGGATTAT	GCTAGCTGGT	AATGCCTTTA	5700
CTGCGGGGAA	GATAATAGTT	TCCTGCATAC	CCCCTGGTTT	TGGTTCACAT	AATCTTACTA	5760
TAGCACAAGC	AACTCTCTTT	CCACATGTGA	TTGCTGATGT	TAGGACTCTA	GACCCCATTG	5820
AGGTGCCTTT	GGAAGATGTT	AGGAATGTTC	TCTTTCATAA	TAATGATAGA	AATCAACAAA	5880
CCATGCGCCT	TGTGTGCATG	CTGTACACCC	CCCTCCGCAC	TGGTGGTGGT	ACTGGTGATT	5940
CTTTTGTAGT	TGCAGGGCGA	GTTATGACTT	GCCCCAGTCC	TGATTTTAAT	TTCTTGTTTT	6000
TAGTCCCTCC	TACGGTGGAG	CAGAAAACCA	GGCCCTTCAC	ACTCCCAAAT	CTGCCATTGA	6060
GTTCTCTGTC	TAACTCACGT	GCCCCTCTCC	CAATCAGTAG	TATGGGCATT	TCCCCAGACA	6120
ATGTCCAGAG	TGTGCAGTTC	CAAAATGGTC	GGTGTACTCT	GGATGGCCGC	CTGGTTGGCA	6180
CCACCCCAGT	TTCATTGTCA	CATGTTGCCA	AGATAAGAGG	GACCTCCAAT	GGCACTGTAA	6240
TCAACCTTAC	TGAATTGGAT	GGCACACCCT	TTCACCCTTT	TGAGGGCCCT	GCCCCCATTG	6300
GGTTTCCAGA	CCTCGGTGGT	TGTGATTGGC	ATATCAATAT	GACACAGTTT	GGCCATTCTA	6360
GCCAGACCCA	GTATGATGTA	GACACCACCC	CTGACACTTT	TGTCCCCCAT	CTTGGTTCAA	6420
TTCAGGCAAA	TGGCATTGGC	AGTGGTAATT	ATGTTGGTGT	TCTTAGCTGG	ATTTCCCCCC	6480

```
CATCACACCC GTCTGGCTCC CAAGTTGACC TTTGGAAGAT CCCCAATTAT GGGTCAAGTA    6540
TTACGGAGGC AACACATCTA GCCCCTTCTG TATACCCCCC TGGTTTCGGA GAGGTATTGG    6600
TCTTTTTCAT GTCAAAAATG CCAGGTCCTG GTGCTTATAA TTTGCCCTGT CTATTACCAC    6660
AAGAGTACAT TTCACATCTT GCTAGTGAAC AAGCCCCTAC TGTAGGTGAG GCTGCCCTGC    6720
TCCACTATGT TGACCCTGAT ACCGGTCGGA ATCTTGGGGA ATTCAAAGCA TACCCTGATG    6780
GTTTCCTCAC TTGTGTCCCC AATGGGGCTA GCTCGGGTCC ACAACAGCTG CCGATCAATG    6840
GGGTCTTTGT CTTTGTTTCA TGGGTGTCCA GATTTTATCA ATTAAAGCCT GTGGGAACTG    6900
CCAGCTCGGC AAGAGGTAGG CTTGGTCTGC GCCGATAATG GCCCAAGCCA TAATTGGTGC    6960
AATTGCTGCT TCCACAGCAG GTAGTGCTCT GGGAGCGGGC ATACAGGTTG GTGGCGAAGC    7020
GGCCCTCCAA AGCCAAAGGT ATCAACAAAA TTTGCAACTG CAAGAAAATT CTTTTAAACA    7080
TGACAGGGAA ATGATTGGGT ATCAGGTTGA AGCTTCAAAT CAATTATTGG CTAAAAATTT    7140
GGCAACTAGA TATTCACTCC TCCGTGCTGG GGGTTTGACC AGTGCTGATG CAGCAAGATC    7200
TGTGGCAGGA GCTCCAGTCA CCCGCATTGT AGATTGGAAT GGCGTGAGAG TGTCTGCTCC    7260
CGAGTCCTCT GCTACCACAT TGAGATCCGG TGGCTTCATG TGAGTTCCCA TACCATTTGC    7320
CTCTAAGCAA AAACAGGTTC AATCATCTGG TATTAGTAAT CCAAATTATT CCCCTTCATC    7380
CATTTCTCGA ACCACTAGTT GGGTCGAGTC ACAAAACTCA TCGAGATTTG GAAATCTTTC    7440
TCCATACCAC GCGGAGGCTC TCAATACAGT GTGGTTGACT CCACCCGGTT CAACAGCCTC    7500
TTCTACACTG TCTTCTGTGC CACGTGGTTA TTTCAATACA GACAGGTTGC CATTATTCGC    7560
AAATAATAGG CGATGATGTT GTAATATGAA ATGTGGGCAT CATATTCATT TAATTAGGTT    7620
TAATTAGGTT TAATTTGATG TTAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    7680
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    7740
AAAAAAAAAA AAA                                                       7753
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1738 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Leu Gly Leu Ile Gly Gln Val Pro Ala Pro Lys Ala Thr Ser
 1               5                  10                  15

Val Asp Val Pro Lys Gln Gln Arg Asp Arg Pro Pro Arg Thr Val Ala
            20                  25                  30

Glu Val Gln Gln Asn Leu Arg Trp Thr Glu Arg Pro Gln Asp Gln Asn
        35                  40                  45

Val Lys Thr Trp Asp Glu Leu Asp His Thr Thr Lys Gln Gln Ile Leu
    50                  55                  60

Asp Glu His Ala Glu Trp Phe Asp Ala Gly Gly Leu Gly Pro Ser Thr
65                  70                  75                  80

Leu Pro Thr Ser His Glu Arg Tyr Thr His Glu Asn Asp Glu Gly His
                85                  90                  95

Gln Val Lys Trp Ser Ala Arg Glu Gly Val Asp Leu Gly Ile Ser Gly
            100                 105                 110

Leu Thr Thr Val Ser Gly Pro Glu Trp Asn Met Cys Pro Leu Pro Pro
        115                 120                 125

Val Asp Gln Arg Ser Thr Thr Pro Ala Thr Glu Pro Thr Ile Gly Asp
    130                 135                 140

Met Ile Glu Phe Tyr Glu Gly His Ile Tyr His Tyr Ala Ile Tyr Ile
145                 150                 155                 160
```

```
Gly Gln Gly Lys Thr Val Gly Val His Ser Pro Gln Ala Ala Phe Ser
                165                 170                 175

Ile Thr Arg Ile Thr Ile Gln Pro Ile Ser Ala Trp Trp Arg Val Cys
            180                 185                 190

Tyr Val Pro Gln Pro Lys Gln Arg Leu Thr Tyr Asp Gln Leu Lys Glu
        195                 200                 205

Leu Glu Asn Glu Pro Trp Pro Tyr Ala Ala Val Thr Asn Asn Cys Phe
    210                 215                 220

Glu Phe Cys Cys Gln Val Met Cys Leu Glu Asp Thr Trp Leu Gln Arg
225                 230                 235                 240

Lys Leu Ile Ser Ser Gly Arg Phe Tyr His Pro Thr Gln Asp Trp Ser
                245                 250                 255

Arg Asp Thr Pro Glu Phe Gln Gln Asp Ser Lys Leu Glu Met Val Arg
            260                 265                 270

Asp Ala Val Leu Ala Ala Ile Asn Gly Leu Val Ser Arg Pro Phe Lys
        275                 280                 285

Asp Leu Leu Gly Lys Leu Lys Pro Leu Asn Val Leu Asn Leu Leu Ser
    290                 295                 300

Asn Cys Asp Trp Thr Phe Met Gly Val Val Glu Met Val Val Leu Leu
305                 310                 315                 320

Leu Glu Leu Phe Gly Ile Phe Trp Asn Pro Pro Asp Val Ser Asn Phe
                325                 330                 335

Ile Ala Ser Leu Leu Pro Asp Phe His Leu Gln Gly Pro Glu Asp Leu
            340                 345                 350

Ala Arg Asp Leu Val Pro Ile Val Leu Gly Gly Ile Gly Leu Ala Ile
        355                 360                 365

Gly Phe Thr Arg Asp Lys Val Ser Lys Met Met Lys Asn Ala Val Asp
    370                 375                 380

Gly Leu Arg Ala Ala Thr Gln Leu Gly Gln Tyr Gly Leu Glu Ile Phe
385                 390                 395                 400

Ser Leu Leu Lys Lys Tyr Phe Phe Gly Gly Asp Gln Thr Glu Lys Thr
                405                 410                 415

Leu Lys Asp Ile Glu Ser Ala Val Ile Asp Met Glu Val Leu Ser Ser
            420                 425                 430

Thr Ser Val Thr Gln Leu Val Arg Asp Lys Gln Ser Ala Arg Ala Tyr
        435                 440                 445

Met Ala Ile Leu Asp Asn Glu Glu Glu Lys Ala Arg Lys Leu Ser Val
    450                 455                 460

Arg Asn Ala Asp Pro His Val Val Ser Ser Thr Asn Ala Leu Ile Ser
465                 470                 475                 480

Arg Ile Ser Met Ala Arg Ala Ala Leu Ala Lys Ala Gln Ala Glu Met
                485                 490                 495

Thr Ser Arg Met Arg Pro Val Val Ile Met Met Cys Gly Pro Pro Gly
            500                 505                 510
```

```
Ile Gly Lys Thr Lys Ala Ala Glu His Leu Ala Lys Arg Leu Ala Asn
        515                 520                 525

Glu Ile Arg Pro Gly Gly Lys Val Gly Leu Val Pro Arg Glu Ala Val
    530                 535                 540

Asp His Trp Asp Gly Tyr His Gly Glu Glu Val Met Leu Trp Asp Asp
545                 550                 555                 560

Tyr Gly Met Thr Lys Ile Gln Glu Asp Cys Asn Lys Leu Gln Ala Ile
                565                 570                 575

Ala Asp Ser Ala Pro Leu Thr Leu Asn Cys Asp Arg Ile Glu Asn Lys
            580                 585                 590

Gly Met Gln Phe Val Ser Asp Ala Ile Val Ile Thr Thr Asn Ala Pro
        595                 600                 605

Gly Pro Ala Pro Val Asp Phe Val Asn Leu Gly Pro Val Cys Arg Arg
    610                 615                 620

Val Asp Phe Leu Val Tyr Cys Thr Ala Pro Glu Val Glu His Thr Arg
625                 630                 635                 640

Lys Val Ser Pro Gly Asp Thr Thr Ala Leu Lys Asp Cys Phe Lys Pro
                645                 650                 655

Asp Phe Ser His Leu Lys Met Glu Leu Ala Pro Gln Gly Gly Phe Asp
            660                 665                 670

Asn Gln Gly Asn Thr Pro Phe Gly Lys Gly Val Met Lys Pro Thr Thr
        675                 680                 685

Ile Asn Arg Leu Leu Ile Gln Ala Val Ala Leu Thr Met Glu Arg Gln
    690                 695                 700

Asp Glu Phe Gln Leu Gln Gly Pro Thr Tyr Asp Phe Asp Thr Asp Arg
705                 710                 715                 720

Val Ala Ala Phe Thr Arg Met Ala Arg Ala Asn Gly Leu Gly Leu Ile
                725                 730                 735

Ser Met Ala Ser Leu Gly Lys Lys Leu Arg Ser Val Thr Thr Ile Glu
            740                 745                 750

Gly Leu Lys Asn Ala Leu Ser Gly Tyr Lys Ile Ser Lys Cys Ser Ile
        755                 760                 765

Gln Trp Gln Ser Arg Val Tyr Ile Ile Glu Ser Asp Gly Ala Ser Val
    770                 775                 780

Gln Ile Lys Glu Asp Lys Gln Ala Leu Thr Pro Leu Gln Gln Thr Ile
785                 790                 795                 800

Asn Thr Ala Ser Leu Ala Ile Thr Arg Leu Lys Ala Ala Arg Ala Val
                805                 810                 815

Ala Tyr Ala Ser Cys Phe Gln Ser Ala Ile Thr Thr Ile Leu Gln Met
            820                 825                 830

Ala Gly Ser Ala Leu Val Ile Asn Arg Ala Val Lys Arg Met Phe Gly
        835                 840                 845

Thr Arg Thr Ala Ala Met Ala Leu Glu Gly Pro Gly Lys Glu His Asn
    850                 855                 860
```

Cys Arg Val His Lys Ala Lys Glu Ala Gly Lys Gly Pro Ile Gly His
865 870 875 880

Asp Asp Met Val Glu Arg Phe Gly Leu Cys Glu Thr Glu Glu Glu Glu
885 890 895

Ser Glu Asp Gln Ile Gln Met Val Pro Ser Asp Ala Val Pro Glu Gly
900 905 910

Lys Asn Lys Gly Lys Thr Lys Lys Gly Arg Gly Arg Lys Asn Asn Tyr
915 920 925

Asn Ala Phe Ser Arg Arg Gly Leu Ser Asp Glu Glu Tyr Glu Glu Tyr
930 935 940

Lys Lys Ile Arg Glu Glu Lys Asn Gly Asn Tyr Ser Ile Gln Glu Tyr
945 950 955 960

Leu Glu Asp Arg Gln Arg Tyr Glu Glu Glu Leu Ala Glu Val Gln Ala
965 970 975

Gly Gly Asp Gly Gly Ile Gly Glu Thr Glu Met Glu Ile Arg His Arg
980 985 990

Val Phe Tyr Lys Ser Lys Ser Lys Lys His Gln Gln Glu Gln Arg Arg
995 1000 1005

Gln Leu Gly Leu Val Thr Gly Ser Asp Ile Arg Lys Arg Lys Pro Ile
1010 1015 1020

Asp Trp Thr Pro Pro Lys Asn Glu Trp Ala Asp Asp Asp Arg Glu Val
1025 1030 1035 1040

Asp Tyr Asn Glu Lys Ile Asn Phe Glu Ala Pro Pro Thr Leu Trp Ser
1045 1050 1055

Arg Val Thr Lys Phe Gly Ser Gly Trp Gly Phe Trp Val Ser Pro Thr
1060 1065 1070

Val Phe Ile Thr Thr Thr His Val Val Pro Thr Gly Val Lys Glu Phe
1075 1080 1085

Phe Gly Glu Pro Leu Ser Ser Ile Ala Ile His Gln Ala Gly Glu Phe
1090 1095 1100

Thr Gln Phe Arg Phe Ser Lys Lys Met Arg Pro Asp Leu Thr Gly Met
1105 1110 1115 1120

Val Leu Glu Glu Gly Cys Pro Glu Gly Thr Val Cys Ser Val Leu Ile
1125 1130 1135

Lys Arg Asp Ser Gly Glu Leu Leu Pro Leu Ala Val Arg Met Gly Ala
1140 1145 1150

Ile Ala Ser Met Arg Ile Gln Gly Arg Leu Val His Gly Gln Ser Gly
1155 1160 1165

Met Leu Leu Thr Gly Ala Asn Ala Lys Gly Met Asp Leu Gly Thr Ile
1170 1175 1180

Pro Gly Asp Cys Gly Ala Pro Tyr Val His Lys Arg Gly Asn Asp Trp
1185 1190 1195 1200

Val Val Cys Gly Val His Ala Ala Ala Thr Lys Ser Gly Asn Thr Val
1205 1210 1215

Val Cys Ala Val Gln Ala Gly Glu Gly Glu Thr Ala Leu Glu Gly Gly
1220 1225 1230

Asp Lys Gly His Tyr Ala Gly His Glu Ile Val Arg Tyr Gly Ser Gly
1235 1240 1245

Pro Ala Leu Ser Thr Lys Thr Lys Phe Trp Arg Ser Ser Pro Glu Pro
1250 1255 1260

Leu Pro Pro Gly Val Tyr Glu Pro Ala Tyr Leu Gly Gly Lys Asp Pro
1265 1270 1275 1280

Arg Val Gln Asn Gly Pro Ser Leu Gln Gln Val Leu Arg Asp Gln Leu
1285 1290 1295

Lys Pro Phe Ala Asp Pro Arg Gly Arg Met Pro Glu Pro Gly Leu Leu
1300 1305 1310

Glu Ala Ala Val Glu Thr Val Thr Ser Met Leu Glu Gln Thr Met Asp
1315 1320 1325

Thr Pro Ser Pro Trp Ser Tyr Ala Asp Ala Cys Gln Ser Leu Asp Lys
1330 1335 1340

Thr Thr Ser Ser Gly Tyr Pro His His Lys Arg Lys Asn Asp Asp Trp
1345 1350 1355 1360

Asn Gly Thr Thr Phe Val Gly Glu Leu Gly Glu Gln Ala Ala His Ala
1365 1370 1375

Asn Asn Met Tyr Glu Asn Ala Lys His Met Lys Pro Ile Tyr Thr Ala
1380 1385 1390

Ala Leu Lys Asp Glu Leu Val Lys Pro Glu Lys Ile Tyr Gln Lys Val
1395 1400 1405

Lys Lys Arg Leu Leu Trp Gly Ala Asp Leu Gly Thr Val Val Arg Ala
1410 1415 1420

Ala Arg Ala Phe Gly Pro Phe Cys Asp Ala Ile Lys Ser His Val Ile
1425 1430 1435 1440

Lys Leu Pro Ile Lys Val Gly Met Asn Thr Ile Glu Asp Gly Pro Leu
1445 1450 1455

Ile Tyr Ala Glu His Ala Lys Tyr Lys Asn His Phe Asp Ala Asp Tyr
1460 1465 1470

Thr Ala Trp Asp Ser Thr Gln Asn Arg Gln Ile Met Thr Glu Ser Phe
1475 1480 1485

Ser Ile Met Ser Arg Leu Thr Ala Ser Pro Glu Leu Ala Glu Val Val
1490 1495 1500

Ala Gln Asp Leu Leu Ala Pro Ser Glu Met Asp Val Gly Asp Tyr Val
1505 1510 1515 1520

Ile Arg Val Lys Glu Gly Leu Pro Ser Gly Phe Pro Cys Thr Ser Gln
1525 1530 1535

Val Asn Ser Ile Asn His Trp Ile Ile Thr Leu Cys Ala Leu Ser Glu
1540 1545 1550

Ala Thr Gly Leu Ser Pro Asp Val Val Gln Ser Met Ser Tyr Phe Ser
1555 1560 1565

```
Phe Tyr Gly Asp Asp Glu Ile Val Ser Thr Asp Ile Asp Phe Asp Pro
    1570                1575                1580

Ala Arg Leu Thr Gln Ile Leu Lys Glu Tyr Gly Leu Lys Pro Thr Arg
1585                1590                1595                1600

Pro Asp Lys Thr Glu Gly Pro Ile Gln Val Arg Lys Asn Val Asp Gly
                1605                1610                1615

Leu Val Phe Leu Arg Arg Thr Ile Ser Arg Asp Ala Ala Gly Phe Gln
            1620                1625                1630

Gly Arg Leu Asp Arg Ala Ser Ile Glu Arg Gln Ile Phe Trp Thr Arg
        1635                1640                1645

Gly Pro Asn His Ser Asp Pro Ser Glu Thr Leu Val Pro His Thr Gln
    1650                1655                1660

Arg Lys Ile Gln Leu Ile Ser Leu Leu Gly Glu Ala Ser Leu His Gly
1665                1670                1675                1680

Glu Lys Phe Tyr Arg Lys Ile Ser Ser Lys Val Ile His Glu Ile Lys
                1685                1690                1695

Thr Gly Gly Leu Glu Met Tyr Val Pro Gly Trp Gln Ala Met Phe Arg
            1700                1705                1710

Trp Met Arg Phe His Asp Leu Gly Leu Trp Thr Gly Asp Arg Asp Leu
        1715                1720                1725

Leu Pro Glu Phe Val Asn Asp Asp Gly Val
    1730                1735
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 530 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Met Met Ala Ser Lys Asp Ala Thr Ser Ser Val Asp Gly
 1               5                  10

Ala Ser Gly Ala Gly Gln Leu Val Pro Glu Val Asn Ala Ser Asp Pro
 15                 20                  25                  30

Leu Ala Met Asp Pro Val Ala Gly Ser Ser Thr Ala Val Ala Thr Ala
                35                  40                  45

Gly Gln Val Asn Pro Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln
            50                  55                  60

Ala Pro Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp
        65                  70                  75

Val Leu Phe Asp Leu Ser Leu Gly Pro His Leu Asn Pro Phe Leu Leu
    80                  85                  90

His Leu Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg
 95                 100                 105                 110
```

```
Ile Met Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Ile Ile Val Ser
                115                 120                 125

Cys Ile Pro Pro Gly Phe Gly Ser His Asn Leu Thr Ile Ala Gln Ala
            130                 135                 140

Thr Leu Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Asp Pro Ile
        145                 150                 155

Glu Val Pro Leu Glu Asp Val Arg Asn Val Leu Phe His Asn Asn Asp
    160                 165                 170

Arg Asn Gln Gln Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu
175                 180                 185                 190

Arg Thr Gly Gly Gly Thr Gly Asp Ser Phe Val Val Ala Gly Arg Val
                195                 200                 205

Met Thr Cys Pro Ser Pro Asp Phe Asn Phe Leu Phe Leu Val Pro Pro
            210                 215                 220

Thr Val Glu Gln Lys Thr Arg Pro Phe Thr Leu Pro Asn Leu Pro Leu
        225                 230                 235

Ser Ser Leu Ser Asn Ser Arg Ala Pro Leu Pro Ile Ser Ser Met Gly
    240                 245                 250

Ile Ser Pro Asp Asn Val Gln Ser Val Gln Phe Gln Asn Gly Arg Cys
255                 260                 265                 270

Thr Leu Asp Gly Arg Leu Val Gly Thr Thr Pro Val Ser Leu Ser His
                275                 280                 285

Val Ala Lys Ile Arg Gly Thr Ser Asn Gly Thr Val Ile Asn Leu Thr
            290                 295                 300

Glu Leu Asp Gly Thr Pro Phe His Pro Phe Glu Gly Pro Ala Pro Ile
        305                 310                 315

Gly Phe Pro Asp Leu Gly Gly Cys Asp Trp His Ile Asn Met Thr Gln
    320                 325                 330

Phe Gly His Ser Ser Gln Thr Gln Tyr Asp Val Asp Thr Thr Pro Asp
335                 340                 345                 350

Thr Phe Val Pro His Leu Gly Ser Ile Gln Ala Asn Gly Ile Gly Ser
                355                 360                 365

Gly Asn Tyr Val Gly Val Leu Ser Trp Ile Ser Pro Pro Ser His Pro
            370                 375                 380

Ser Gly Ser Gln Val Asp Leu Trp Lys Ile Pro Asn Tyr Gly Ser Ser
        385                 390                 395

Ile Thr Glu Ala Thr His Leu Ala Pro Ser Val Tyr Pro Pro Gly Phe
    400                 405                 410

Gly Glu Val Leu Val Phe Phe Met Ser Lys Met Pro Gly Pro Gly Ala
415                 420                 425                 430

Tyr Asn Leu Pro Cys Leu Leu Pro Gln Glu Tyr Ile Ser His Leu Ala
                435                 440                 445

Ser Glu Gln Ala Pro Thr Val Gly Glu Ala Ala Leu Leu His Tyr Val
            450                 455                 460
```

```
Asp Pro Asp Thr Gly Arg Asn Leu Gly Glu Phe Lys Ala Tyr Pro Asp
        465                 470                 475

Gly Phe Leu Thr Cys Val Pro Asn Gly Ala Ser Ser Gly Pro Gln Gln
    480                 485                 490

Leu Pro Ile Asn Gly Val Phe Val Phe Val Ser Trp Val Ser Arg Phe
495                 500                 505                 510

Tyr Gln Leu Lys Pro Val Gly Thr Ala Ser Ser Ala Arg Gly Arg Leu
                515                 520                 525

Gly Leu Arg Arg
            530
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
                                        Met Ala Gln Ala Ile Ile
                                          1               5

Gly Ala Ile Ala Ala Ser Thr Ala Gly Ser Ala Leu Gly Ala Gly Ile
            10                  15                  20

Gln Val Gly Gly Glu Ala Ala Leu Gln Ser Gln Arg Tyr Gln Gln Asn
        25                  30                  35

Leu Gln Leu Gln Glu Asn Ser Phe Lys His Asp Arg Glu Met Ile Gly
    40                  45                  50

Tyr Gln Val Glu Ala Ser Asn Gln Leu Leu Ala Lys Asn Leu Ala Thr
55                  60                  65                  70

Arg Tyr Ser Leu Leu Arg Ala Gly Gly Leu Thr Ser Ala Asp Ala Ala
                75                  80                  85

Arg Ser Val Ala Gly Ala Pro Val Thr Arg Ile Val Asp Trp Asn Gly
            90                  95                 100

Val Arg Val Ser Ala Pro Glu Ser Ser Ala Thr Thr Leu Arg Ser Gly
        105                 110                 115

Gly Phe Met Ser Val Pro Ile Pro Phe Ala Ser Lys Gln Lys Gln Val
    120                 125                 130

Gln Ser Ser Gly Ile Ser Asn Pro Asn Tyr Ser Pro Ser Ser Ile Ser
135                 140                 145                 150

Arg Thr Thr Ser Trp Val Glu Ser Gln Asn Ser Ser Arg Phe Gly Asn
                155                 160                 165

Leu Ser Pro Tyr His Ala Glu Ala Leu Asn Thr Val Trp Leu Thr Pro
            170                 175                 180

Pro Gly Ser Thr Ala Ser Ser Thr Leu Ser Ser Val Pro Arg Gly Tyr
        185                 190                 195
```

```
Phe Asn Thr Asp Arg Leu Pro Leu Phe Ala Asn Asn Arg Arg
    200                 205                 210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 551 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human calicivirus Sapporo

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..549

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TGTGATGCTG CCACCACGCT TATAGCCACC GCGGCTTTTA AGGCCGTGGC TACNAGGCTA     60

CAGGTGGTGA CACCAATGAC ACCAGTTGCT GTTGGCATTA ACATGGACTC TGTTCAGATG    120

CAAGTGATGA ATGACTCTTT AAAGGGGGGT GTTCTTTACT GTTTGGATTA TTCCAAATGG    180

GATTCCACAC AAAACCCTGC AGTGACAGCA GCCTCCCTGG CAATATTGGA GAGATTTGCT    240

GAGCCCCATC CAATTGTGTC TTGTGCCATT GAGGCTCTTT CCTCCCCTGC AGAGGGCTAT    300

GTCAATGATA TCAAATTTGT GACACGCGGC GGCCTACCAT CTGGGATGCC ATTTACATCT    360

GTCGTCAATT CTATCAACCA TATGATATAC GTGGCGGCAG CCATCCTGCA GGCATACGAA    420

AGCCACAATG TCCCATATAC TGGAAACGTC TTCCAAGTGG AGACCGTTCA CACGTATGGT    480

GATGATTGCA TGTACAGCGT GTGCCCTGCC ACTGCATCAA TTTTCCACAC TGTGCTTGCA    540

AACCTAACGT C                                                         551
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Cys Asp Ala Ala Thr Thr Leu Ile Ala Thr Ala Ala Phe Lys Ala Ala
1               5                   10                  15

Val Xaa Arg Leu Gln Val Val Thr Pro Met Thr Pro Val Ala Val Gly
            20                  25                  30

Ile Asn Met Asp Ser Val Gln Met Gln Val Met Asn Asp Ser Leu Lys
        35                  40                  45
```

```
Gly Gly Val Leu Tyr Cys Leu Asp Tyr Ser Lys Trp Asp Ser Thr Gln
    50                  55                  60

Asn Pro Ala Val Thr Ala Ala Ser Leu Ala Ile Leu Glu Arg Phe Ala
65                  70                  75                      80

Glu Pro His Pro Ile Val Ser Cys Ala Ile Glu Ala Leu Ser Ser Pro
                85                  90                      95

Ala Glu Gly Tyr Val Asn Asp Ile Lys Phe Val Thr Arg Gly Gly Leu
            100                 105                 110

Pro Ser Gly Met Pro Phe Thr Ser Val Val Asn Ser Ile Asn His Met
        115                 120                 125

Ile Tyr Val Ala Ala Ala Ile Leu Gly Ala Tyr Glu Ser His Asn Val
    130                 135                 140

Pro Tyr Thr Gly Asn Val Phe Gln Val Glu Thr Val His Thr Tyr Gly
145                 150                 155                     160

Asp Asp Cys Met Tyr Ser Val Cys Pro Ala Thr Ala Ser Ile Phe His
                165                 170                     175

Thr Val Leu Ala Asn Leu Thr
            180
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 148 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
TGTGATGCTG CCACCACGCT TATAGCCACC GCGGCTTTTA AGGCCGTGGC TACAGGCTAC    60

AGGTGGTGAC ACCAATGACA CCAGTTGCTG TTGGCATTAA CATGGACTCT GTTCAGATGC   120

AAGTGATGAA TGACTCTTTA AAGGGGGG                                      148
```

(2) INFORMATION FOR SEQ ID NO:8
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
ATGGACTCTG TTCAGATGCA AGTGATGAAT GACTCTTTAA AGGGGGGTGT TCTTTACTGT    60

TTGGATTATT CCAAATGGGA TTCCACACAA AACCCTGCAG TGACAGCAGC CTCCCTGGCA   120

ATATTGGAGA GATTTGCTGA GCCCCATCCA ATTGTGTCTT GTGCCATTGA GGCTCTTTCC   180

TCCCCTGCAG AGGGCTATGT CAATGATATC AAATTTGTGA CACGCGGCGG CCTACCATCT   240
```

GGGATGCCAT TTACATCTGT CGTCAATTCT ATCAACCATA TGATATACGT GGCGGCAGCC 300

ATCCTGCAGG CATACGAAAG CCACAATGTC CCATATACTG GAAACGTCTT CCAAGTGGAG 360

ACCGTTCACA CGTATGGTGA TGATTGCATG TACAGCGTGT GCCCTGCCAC TGCATCAATT 420

TTCCACACTG TGCTTGCAAA CCTAACGTC 449

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 446 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human calicivirus Saporro (Day care)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGGACTCTG TTCAGATGCA AGTGATGAAT GACTCTTTAA AGGGAGGTGT TCTCTACTGC 60

CTGGATTACT CCAAATGGGA CTCCACACAA AATGCTGCAG TGACAGCAGC ATCCCTNNCA 120

ATATTGGAGA GATTTGCTGA ACCCCACCCA ATTGTGTCTT GTGCCATTGA GGCCCTGNNC 180

TCNNCTGCAG AGGGTTACGT TAATGATATC AAGTTTGTGA CACGTGGCGG CCTACCATGT 240

GGGATGCCAT TCACATCTGT TGTCAATTCC ATCAACCACA TNATATACGT GGCAGCCGCC 300

ATCCTGCAGG CATACGAAAG CCACAATGTT CCATACACTG GAAATGTCTT CCAAGTGGAG 360

ACTGTTCACA CGTATGGTGA CGATTGCATG TACAGCGTGT GCCCTGCCAC CGCATCAATT 420

TTCCACACTG TACTTGCAAA CCTAAC 446

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 434 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human calicivirus Houston

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..434

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCCATGTTA TAGTGGTGTT CACATGAAAG ATGGCGACAA GATGTTGATA GATGCCAATC 60

TTCCTTACAA CCAGAAATTA ACTACTATGA TTCATGAGAC TAGGCATAGG ATAGGACAGT 120

ATATAGATAA TACTTTTGGA AAGACATTTA GACATGGATT GACAAAACCT GCTGACAAGA 180

CTGTAGATTT GATCTATAAG ACATTGAATT ATGATGATTT TCTGGCAATA ATGCTAATCA 240

TATATGGGCA AAAGTCGGCC ACTAATACGG AGTTGCAATT CTTGATGGAG AAACTTAGAG 300

GTTATGAATC TACAATGGAT GACATAGGGA AAGTCTATGG AGATGATAAA ATGAGAGATA 360

TAATCAAGAA TATTTCTGAT GATGACATAA AGAGTCTTTT AGGGGAGATA AATAGTGATT 420

ATTCTGGTAA GNAT 434

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Cys Tyr Ser Gly Val His Met Lys Asp Gly Asp Lys Met Leu Ile
1 5 10 15

Asp Ala Asn Leu Pro Tyr Asn Gln Lys Leu Thr Thr Met Ile His Glu
20 25 30

Thr Arg His Arg Ile Gly Gln Tyr Ile Asp Asn Thr Phe Gly Lys Thr
35 40 45

Phe Arg His Gly Leu Thr Lys Pro Ala Asp Lys Thr Val Asp Leu Ile
50 55 60

Tyr Lys Thr Leu Asn Tyr Asp Asp Phe Leu Ala Ile Met Leu Ile Ile
65 70 75 80

Tyr Gly Gln Lys Ser Ala Thr Asn Thr Glu Leu Gln Phe Leu Met Glu
85 90 95

Lys Leu Arg Gly Tyr Glu Ser Thr Met Asp Asp Ile Gly Lys Val Tyr
100 105 110

Gly Asp Asp Lys Met Arg Asp Ile Ile Lys Asn Ile Ser Asp Asp Asp
115 120 125

Ile Lys Ser Leu Leu Gly Glu Ile Asn Ser Asp Tyr Ser Gly Lys Xaa
130 135 140

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2516 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/KY/89

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAATAGAGGA TGGCCCTTTA ATTTATGCTG AACATGCCAA GTACAAAAAT CATTTTGATG 60
CAGATTACAC AGCATGGGAC TCTACACAAA ATAGACAAAT TATGACAGAA TCCTTCTCCA 120
TCATGTCACG CCTTACGGCC TCTCCAGAAC TAGCTGAGGT TGTAGCCCAG GACTTACTAG 180
CACCATCCGA GATGGATGTG GGCGACTATG TTATAAGGGT CAAAGAAGGC CTACCATCAG 240
GATTTCCCTG CACTTCTCAA GTGAATAGCA TAAATCACTG GATAATCACC CTTTGTGCAT 300
TGTCTGAGGC TACTGGCTTA TCACCTGATG TGGTACAGTC CATGTCATAC TTCTCATTCT 360
ACGGTGATGA TGAGATCGTA TCAACTGACA TAGACTTTGA CCCAACTCGC CTCACCCAAA 420
TTCTCAAGGA ATACGGCCTC AAGCCAACAA GGCCAGACAA AACAGAAGGA CCAATACAGG 480
TGAGGAAGAA TGTGGATGGG CTAGTTTTTC TGCGGCGCAC CATCTCCCGG GACGCAGCAG 540
GGTTCCAAGG TAGACTGGAT AGAGCCTCAA TTGAACGTCA AATTTTCTGG ACCCGCGGGC 600
CCAACCATTC AGACCCATCA GAGACTCTGG TACCACACAC CCAAAGGAAA GTCCAGCTGA 660
TCTCACTTCT AGGAGAAGCC TCACTCCACG GGGAAAAATT TTACAGGAAA ATATCTAGCA 720
AAGTCATACA TGAAATTAAG ACTGGTGGGC TGGAGATGTA TGTCCCAGGG TGGCAGGCCA 780
TGTTCCGCTG GATGCGCTTC CATGACCTCG GATTGTGGAC AGGAGATCGC AATCTCCTGC 840
CCGAATTCGT AAATGATGAT GGCGTCTAAG GACGCTACGT CAAGCGTGGA TGGCGCCAGT 900
GCGTCGGTTC AGTTGGTACC GGAGGTTAAT GCTTCTGACC CTCTTGCAAT GGATCCTGTG 960
GCGGGTTCTT CAACAGCAGT TGCAACCGCT GGACAAGTTA ACCCTATTGA CCCTTGGATA 1020
ATCAATAACT TTGTGCAGGC TCCCCAAGGT GAATTTACTA TTTCTCCAAA TAATACCCCC 1080
GGTGATGTTT TGTTTGATTT GAGTCTAGGC CCTCATCTTA ATCCCTTCTT GTTACATTTG 1140
TCACAAATGT ATAATGGCTG GGTTGGCAAC ATGAGAGTTA GGATTATGCT GGCTGGTAAT 1200
GCATTTACTG CAGGCAAAAT TATAGTTTCT TGCATACCTC CTGGCTTTGG CTCCCAACAA 1260
CTTACTATAG CACAAGCAAC TCTCTTCCCG CATGTGATTG CTGATGTTAG GACTTTAGAC 1320
CCAATTGAAG TACCCTTGGA AGATGTAAGG AATGTTCTCT TTCATAATAA TGATAGAAAT 1380
CAACAAACTA TGCGCCTTGT GTGCATGCTT TATACCCCCC TCAGCACTGG TGGCGGTACA 1440
GGTGATTCTT TTGTGGTTGC AGGGCGAGTC ATGACTTGTC CTAGCCCCGA CTTTAATTTC 1500
TTGTTCTTGG TTCCTCCCAC AGTGGAACAG AAGACTAGGC CTTTCACCCT CCCAAATTTA 1560
CCGCTGAGTT CTTTGTCTAA TTCACGTGCT CCTCTTCCAA TTAGTGGCAT GGGTATTTCT 1620
CCAGATAATG TTCAGAGTGT GCAGTTCCAA AATGGCCGAT GTACCTTAGA TGGACGTCTT 1680
GTTGGCACCA CCCCAGTTTC CCTCTCCCAT GTTGCTAAGA TAAGGGGTAC TTCTAATGGT 1740
ACAGTAATCA ATCTCACCGA ATTGGATGGC ACCCCCTTCC ACCCTTTTGA AGGCCCTGCC 1800
CCTATTGGTT TTCCAGATCT TGGTGGCTGT GATTGGCATA TTAATATGAC ACAATTTGGA 1860
CATTCCAGTC AGACTCAGTA TGATGTAGAC ACCACCCCCG ACACCTCCGT CCCTCACTTA 1920
GGTTCAATCC AGGCGAATGG CATTGGTAGT GGCAACTATA TTGGTGTTCT TAGCTGGGTC 1980

TCCCCCCCAT CACATCCATC TGGCTCTCAA GTTGATCTCT GGAAGATCCC CAACTATGGG 2040

TCTAGTATCA CAGAGGCAAC CCATCTAGCT CCCTCTGTCT ATTCTCCTGG CTTTGGAGAG 2100

GTGCTAGTCT TTTTCATGTC AAAGATACCA GGTCCTGGTG GTGATAGTCT GCCCTGTTTA 2160

CTGCCACAAG GATATATCTC ACACCTTGCA AGTGAACAAG CCCCAACTGT TGGTGAGGGT 2220

CCCCTGCTCC ACTATGTTGA CCCTGACACG GACCGGAATC TTGGGGAGTT TAAGGCTTAC 2280

CCTGATGGTT TCCTAACCTG TGTCCCTAAT GGGGCCAGCT CGGGCCCACA ACAACTACCA 2340

ATCAATGGAG TCTTTGTCTT TGTTTCATGG GTGTCCAGAT TTTATCAGTT AAAGCCTGTG 2400

GGAACTGCCA GTACGGCAAG AGGTAGGCTT GGTTTGCGCC GATAATGGCT CAGGCTATAA 2460

TTGGTGCAAT TGCCGCCTCT ACAGCAGGTA GTGCTTTAGG GGCAGGTATA CAGGTT 2516

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: primate calcicvirus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGACGGACC TGCTGTTGAA GATCTCTTCA AAGGCTCGAA CGACCAAAGC ACGATCGGTA 60

TTGTGTTGAC TACGCAAAGT GGGACTCAAC CCACCACCAA AAGTAACATC CAATCAATGA 120

CATC 124

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: primate calcicvirus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGAATGACA TCTTCGACTC GATGGACCTA TTCACATATG GTGATGACGG TGTCTACATC 60

GTCCCACCAC TATATCATCT GTCATGCCCA AGTCTTCACC AACCTGAAAC 110

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGTTGGTT TGAGGCCATA T 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATAAAAGTTG GCATGAACA 19

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTTGACACAA TCTCATCATC 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGCCTGCCAT CTGGATTGCC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGCCCCCTG GTATAGGTAA 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TGGTGATGAC TATAGCATCA GACACAAA 28

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ACTCACCCAA ATCCTCCA 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTTCTGACCA CCTAACCT 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGTTTGGGTC CCCATCTTAA TCCTTT 26

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGAACCAAAA CCAGGGGG 18

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AGCAAAGTCA TACATGAAAT 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCATTATACA TTTGTAG 17

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATTATAGTTT CTTGCATA 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CACACTCTGG ACATTGTCTG 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CATTGGGTTT CCAGACCTA 19

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATAATTGGGG ATCTTCCAAA 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TAGTGGCATG GGTATTTC 18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TATGCCAATC ACAGCCAC 18

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCTGGCTCC CAAGTTGACC 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGTATCAGG GTCAACAT 18

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGAGGCTGCC CTGCTCCA 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCACCGCTGT CCGGGAGG 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTGCTGTTG GCATTAACA 19

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE
(A) ORGANISM: Norwalk virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
His Phe Asp Ala Asp Tyr Thr Ala Trp Asp Ser Thr Gln Asn Arg Gln
1               5                   10                  15

Ile Met Thr Glu Ser Phe Ser Ile Met Ser Arg Leu Thr Ala Ser Pro
            20                  25                  30

Glu Leu Ala Glu Val Val Ala Gln Asp Leu Leu Ala Pro Ser Glu Met
        35                  40                  45

Asp Val Gly Asp Tyr Val Ile Arg Val Lys Glu Gly Pro Ser Gly Phe
    50                  55                  60

Pro Cys Thr Ser Gln Val Asn Ser Ile Asn His Trp Ile Ile Thr Leu
65                  70                  75                  80

Cys Ala Leu Ser Glu Ala Thr Gly Leu Ser Pro Asp Val Val Gln Ser
                85                  90                  95

Met Ser Tyr Phe Ser Phe Tyr Gly Asp Asp Glu Ile Val Ser Thr Asp
            100                 105                 110

Ile Asp Phe Asp Pro Ala Arg Leu Thr Gln Ile Leu Lys Glu
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: hepatitis E virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Val Phe Glu Asn Asp Phe Ser Glu Phe Asp Ser Thr Gln Asn Asn Phe
1               5                   10                  15

Ser Leu Gly Leu Glu Cys Ala Ile Met Glu Glu Cys Gly Met Pro Gln
            20                  25                  30

Trp Leu Ile Arg Leu Tyr His Leu Ile Arg Ser Ala Trp Ile Leu Gln
        35                  40                  45

Ala Pro Lys Glu Ser Leu Arg Gly Phe Trp Lys Lys His Ser Lys His
    50                  55                  60

Ser Gly Glu Pro Gly Thr Leu Leu Trp Asn Thr Val Trp Asn Met Ala
65                  70                  75                  80

Val Ile Thr His Cys Tyr Asp Phe Arg Asp Phe Gln Val Ala Ala Phe
                85                  90                  95

Lys Gly Asp Asp Ser Ile Val Leu Cys Ser Glu Tyr Arg Gln Ser Pro
            100                 105                 110

Gly Ala Ala Val Leu Ile Ala Gly Cys
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: hepatitis C virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser
1               5                   10                  15

Asp Ile Arg Thr Glu Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro
            20                  25                  30

Gln Ala Arg Val Ala Ile Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly
        35                  40                  45

Gly Pro Leu Thr Asn Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys
    50                  55                  60

Arg Ala Ser Arg Ala Ser Gly Val Leu Thr Thr Ser Cys Gly Asn Thr
65                  70                  75                  80

Leu Thr Cys Tyr Ile Lys Ala Arg Ala Ala Cys Arg Ala Ala Gly Leu
                85                  90                  95

Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Cys
            100                 105                 110

Glu Ser Ala Gly Val Gln Glu Asp Ala Ala Ser Leu Arg Ala Phe
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: hepatitis A virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Gly Leu Asp Leu Asp Phe Ser Ala Phe Asp Ala Ser Leu Ser Pro Phe
1               5                   10                  15

Met Ile Arg Glu Ala Gly Arg Ile Met Ser Glu Leu Ser Gly Thr Pro
            20                  25                  30

Ser His Phe Gly Thr Ala Leu Ile Asn Thr Ile Ile Tyr Ser Lys His
        35                  40                  45

Leu Leu Tyr Asn Cys Cys Tyr His Val Cys Gly Ser Met Pro Ser Gly
    50                  55                  60

Ser Pro Cys Thr Ala Leu Leu Asn Ser Ile Ile Asn Asn Val Asn Leu
65                  70                  75                  80

Tyr Tyr Val Phe Ser Lys Ile Phe Gly Lys Ser Pro Val Phe Phe Cys
                85                  90                  95

Gln Ala Leu Lys Ile Leu Cys Tyr Gly Asp Asp Val Leu Ile Val Phe
            100                 105                 110

Ser Arg Asp Val Gln Ile Asp Asn Leu Asp Leu Ile Gly Gln Lys Ile
        115                 120                 125

Val Asp Glu Phe
    130
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 158 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Japanese encephalitis virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp Thr Arg Ile Thr Arg Thr
1               5                   10                  15

Asp Leu Glu Asn Glu Ala Lys Val Leu Glu Leu Leu Asp Gly Glu His
            20                  25                  30

Arg Met Leu Ala Arg Ala Ile Ile Glu Leu Thr Tyr Arg His Lys Val
        35                  40                  45
```

Val Lys Val Met Arg Pro Ala Ala Glu Gly Lys Thr Val Met Asp Val
50 55 60

Ile Ser Arg Glu Asp Gln Arg Gly Ser Gly Gln Val Val Thr Tyr Ala
65 70 75 80

Leu Asn Thr Phe Thr Asn Ile Ala Val Gln Leu Val Arg Leu Met Glu
85 90 95

Ala Glu Gly Val Ile Gly Pro Gln His Leu Glu Gln Leu Pro Arg Lys
100 105 110

Thr Lys Ile Ala Val Arg Thr Trp Leu Phe Glu Asn Gly Glu Glu Arg
115 120 125

Val Thr Arg Met Ala Ile Ser Gly Asp Asp Cys Val Val Lys Pro Leu
130 135 140

Asp Asp Arg Phe Ala Thr Ala Leu His Phe Leu Asn Ala Met
145 150 155

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Poliovirus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Phe Ala Phe Asp Tyr Thr Gly Tyr Asp Ala Ser Leu Ser Pro Ala Trp
1 5 10 15

Phe Glu Ala Leu Lys Met Val Leu Glu Lys Ile Gly Phe Gly Asp Arg
20 25 30

Val Asp Tyr Ile Asp Tyr Leu Asn His Ser His His Leu Tyr Lys Asn
35 40 45

Lys Thr Tyr Cys Val Lys Gly Gly Met Pro Ser Gly Cys Ser Gly Thr
50 55 60

Ser Ile Phe Asn Ser Met Ile Asn Asn Leu Ile Ile Arg Thr Leu Leu
65 70 75 80

Leu Lys Thr Tyr Lys Gly Ile Asp Leu Asp His Leu Lys Met Ile Ala
85 90 95

Tyr Gly Asp Asp Val Ile Ala Ser Tyr Pro His Glu Val Asp Ala Ser
100 105 110

Leu Leu Ala Gln Ser
115

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 121 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Foot-and-mouth disease virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Val Trp Asp Val Asp Tyr Ser Ala Phe Asp Ala Asn His Cys Ser Asp
1               5                   10                  15

Ala Met Asn Ile Met Phe Glu Glu Val Phe Arg Thr Asp Phe Gly Phe
            20                  25                  30

His Pro Asn Ala Glu Trp Ile Leu Lys Thr Leu Val Asn Thr Glu His
        35                  40                  45

Ala Tyr Glu Asn Lys Arg Ile Thr Val Glu Gly Gly Met Pro Ser Gly
    50                  55                  60

Cys Ser Ala Thr Ser Ile Ile Asn Thr Ile Leu Asn Asn Ile Tyr Val
65                  70                  75                  80

Leu Tyr Ala Leu Arg Arg His Tyr Glu Gly Val Glu Leu Asp Thr Tyr
                85                  90                  95

Thr Met Ile Ser Tyr Gly Asp Asp Ile Val Val Ala Ser Asp Tyr Asp
            100                 105                 110

Leu Asp Phe Glu Ala Leu Lys Pro His
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: encephalomyocarditis virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Val Tyr Asp Val Asp Tyr Ser Asn Phe Asp Ser Thr His Ser Val Ala
1               5                   10                  15

Met Phe Arg Leu Leu Ala Glu Glu Phe Phe Thr Pro Glu Asn Gly Phe
            20                  25                  30

Asp Pro Leu Thr Arg Glu Tyr Leu Glu Ser Leu Ala Ile Ser Thr His
        35                  40                  45

Ala Phe Glu Glu Lys Arg Phe Leu Ile Thr Gly Gly Leu Pro Ser Gly
    50                  55                  60

Cys Ala Ala Thr Ser Met Leu Asn Thr Ile Met Asn Asn Ile Ile Ile
65                  70                  75                  80
```

```
Arg Ala Gly Leu Tyr Leu Thr Tyr Lys Asn Phe Glu Phe Asp Asp Val
                85                  90                  95

Lys Val Leu Ser Tyr Gly Asp Asp Leu Leu Val Ala Thr Asn Tyr Gln
            100                 105                 110

Leu Asp Phe Asp Lys Val Arg Ala Ser Leu Ala Lys Thr Gly
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Sindbis virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
Val Leu Glu Thr Asp Ile Ala Ser Phe Asp Lys Ser Gln Asp Asp Ala
1               5                   10                  15

Met Ala Leu Thr Gly Leu Met Ile Leu Glu Asp Leu Gly Val Asp Gln
            20                  25                  30

Pro Leu Leu Asp Leu Ile Glu Cys Ala Phe Gly Glu Ile Ser Ser Thr
        35                  40                  45

His Leu Pro Thr Gly Thr Arg Phe Lys Phe Gly Ala Met Met Lys Ser
    50                  55                  60

Gly Met Phe Leu Thr Leu Phe Val Asn Thr Val Leu Asn Val Val Ile
65                  70                  75                  80

Ala Ser Arg Val Leu Glu Glu Arg Leu Lys Thr Ser Arg Cys Ala Ala
                85                  90                  95

Phe Ile Gly Asp Asp Asn Ile Ile His Gly Val Val Ser Asp Lys Glu
            100                 105                 110

Met Ala Glu Arg Cys Ala Thr Trp Leu Asn
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: tobacco mosaic virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

```
Val Leu Glu Leu Asp Ile Ser Lys Tyr Asp Lys Ser Gln Asn Glu Phe
1               5                   10                  15
```

```
His Cys Ala Val Glu Tyr Glu Ile Trp Arg Arg Leu Gly Phe Glu Asp
            20                  25                  30

Phe Leu Gly Glu Val Trp Lys Gln Gly His Arg Lys Thr Thr Leu Lys
        35                  40                  45

Asp Ile Thr Ala Gly Tyr Lys Thr Cys Ile Trp Tyr Gln Arg Lys Ser
    50                  55                  60

Gly Asp Val Thr Thr Phe Ile Gly Asn Thr Val Ile Ile Ala Ala Cys
65                  70                  75                  80

Leu Ala Ser Met Leu Pro Met Glu Lys Ile Ile Lys Gly Ala Phe Cys
                85                  90                  95

Gly Asp Asp Ser Leu Leu Tyr Phe Pro Lys Gly Cys Glu Phe Pro Asp
            100                 105                 110

Val Gln His Ser Ala Asn Leu Met Trp Asn Phe Glu
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: alfalfa mosaic virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
Phe Lys Glu Ile Asp Phe Ser Lys Phe Asp Lys Ser Gln Asn Glu Leu
1               5                   10                  15

His His Leu Ile Gln Glu Arg Phe Leu Lys Tyr Leu Gly Ile Pro Asn
            20                  25                  30

Glu Phe Leu Thr Leu Trp Phe Asn Ala His Arg Lys Ser Arg Ile Ser
        35                  40                  45

Asp Ser Lys Asn Gly Val Phe Phe Asn Val Asp Phe Gln Arg Arg Thr
    50                  55                  60

Gly Asp Ala Leu Thr Tyr Leu Gly Asn Thr Ile Val Thr Leu Ala Cys
65                  70                  75                  80

Leu Cys His Val Tyr Asp Leu Met Asp Pro Asn Val Lys Phe Val Val
                85                  90                  95

Ala Ser Gly Asp Asp Ser Leu Ile Gly Thr Val Glu Glu Leu Pro Arg
            100                 105                 110

Asp Gln Glu Phe Leu Phe Thr Thr Leu Phe Asn Leu Glu
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: brome mosaic virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```
Phe Leu Glu Ala Asp Leu Ser Lys Phe Asp Lys Ser Gln Gly Glu Leu
1               5                   10                  15

His Leu Glu Phe Gln Arg Glu Ile Leu Leu Ala Leu Gly Phe Pro Ala
            20                  25                  30

Pro Leu Thr Asn Trp Trp Ser Asp Phe His Arg Asp Ser Tyr Leu Ser
        35                  40                  45

Asp Pro His Ala Lys Val Gly Met Ser Val Ser Phe Gln Arg Arg Thr
    50                  55                  60

Gly Asp Ala Phe Thr Tyr Phe Gly Asn Thr Leu Val Thr Met Ala Met
65                  70                  75                  80

Ile Ala Tyr Ala Ser Asp Leu Ser Asp Cys Asp Cys Ala Ile Phe Ser
                85                  90                  95

Gly Asp Asp Ser Leu Ile Ile Ser Lys Val Lys Pro Val Leu Asp Thr
            100                 105                 110

Asp Met Phe Thr Ser Leu Phe Asn Met Glu
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 142 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: cowpea mosaic virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Val Leu Cys Cys Asp Tyr Ser Ser Phe Asp Gly Leu Leu Ser Lys Gln
1               5                   10                  15

Val Met Asp Val Ile Ala Ser Met Ile Asn Glu Leu Cys Gly Gly Glu
            20                  25                  30

Asp Gln Leu Lys Asn Ala Arg Arg Asn Leu Leu Met Ala Cys Cys Ser
        35                  40                  45

Arg Leu Ala Ile Cys Lys Asn Thr Val Trp Arg Val Glu Cys Gly Ile
    50                  55                  60
```

Pro Ser Gly Phe Pro Met Thr Val Ile Val Asn Ser Ile Phe Asn Glu
65 70 75 80

Ile Leu Ile Arg Tyr His Tyr Lys Lys Leu Met Arg Glu Gln Gln Ala
85 90 95

Pro Glu Leu Met Val Gln Ser Phe Asp Lys Leu Ile Gly Leu Val Thr
100 105 110

Tyr Gly Asp Asp Asn Leu Ile Ser Val Asn Ala Val Val Thr Pro Tyr
115 120 125

Phe Asp Gly Lys Lys Leu Lys Gln Ser Leu Ala Gln Gly Gly
130 135 140

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CACGCGGAGG CTCTCAAT 18

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GGTGGCGAAG CGGCCCTC 18

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TCAGCAGTTA TAGATATG 18

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ATGCTATATA CATAGGTC 18

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CAACAGGTAC TACGTGAC 18

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGTGGCCCAA GATTTGCT 18

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATAAAAGTTG GCATGAACAC AAAT 24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GTTGCTGTTG GCATTAACAT GGAC 24

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GTTCCTGTTG GCATAAACAT GGAC 24

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GTTCCGGTTG GCATTAACAT GGAC 24

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GTTCCGGTTG GTATCAACAT GGAC 24

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GTTGCGGTTG GTGTTGACAT GACA 24

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/CDC 6/91

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

ATGCACTTCA CAGGTGAATA GCATCAACCA CTGGATCCTA ACTCTATGTG CATTGTCAGA 60

AGTCACTGGC TTGTCCCCTG ATGTGATACA ATCACAATCT TATTTCTCAT TTTATGGT 118

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/UT/88

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ATGTACCTCA CAAGTGAACA GCATCAATCA CTGGATTTTG ACCTTGTGGG GCCTATCAGA 60

AGTTACTGGT CTGGCTCCTG ATGTAATACA GTCACAATCT TACTTTTCAT TCTATGGT 118

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Snow Mountain Agent/78

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CTGCACATCA CAGTGGAATT CCATGCCCAC TGGCTCCTCA CACTCTGTGC ACTATCTGAA 60

GTCACAAACC TGGCTCCTGA CATCATACAA GCTAACTCCT TGTTCTCTTT CTATGGT 117

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/CAMBRIDGE, UK 92

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

CTGCACCTCA CAGTGGAACT CCATTGCCCA CTGGTTGCTT ACTCTGTGTG CCCTTTCTGA 60

AGTGACAGGA CTAGGCCCCG ACATCATACA AGCTAATTCC ATGTACTCTT TCTATGGT 118

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/CDC 32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TTGCACCTCA CAGTGGAACT CCATTGCCCT CTGGTTGCTT ACTCTGTGTG CCCTTTCTGA 60

AGTGACAGGA CTAGGCCCCG ACATCATACA AGCTAATTCC ATGTACTCTT TCTATGGT 118

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Norwalk virus/8FIIa/68

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

ATGTACTTCC CAGGTGAACA GCATAAATCA CTGGATAATT ACTCTCTGTG CACTGTCTGA 60

GGCCACTGGT TTATCACCTG ATGTGGTGCA ATCCATGTCA TATTTCTCAT TTTATGGT 118

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV-3/88

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTGCACTTCT CAAGTAAATA GCATAAATCA CTGGATAATC ACCCTTTGTG CACTGTCTGA 60

GGCTACTGGC TTATCACCTG ATGTGGTGCA GTCCATGTCA TACTTCTCAT TTTACGGT 118

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/KY89/89

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTGCACTTCT CAAGTGAATS GCATAAATCA CTGGATAATC ACCCTTTGTG CATTGTCTGA 60

GGCTACTGGC TTATCACCTG ATGTGGTACA GTCCATGTCA TACTTCTCAT TCTACGGT 118

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Norwalk Virus/8FIIa/68

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CAATAGAAGA TGGCCCCCTC ATCTATGCTG AGCATGCTAA ATATAAGAAT CATTTTGATG 60

CAGATTATAC AGCATGGGAC TCAACACAAA ATAGACAAAT TATGACAGAA TCCTTCTCCA 120

TTATGTCGCG CCTTACGGCC TCACCAGAAT TGGCCGAGGT TGTGGCCCAA GATTTGCTAG 180

CACCATCTGA GATGGATGTA GGTGATTATG TCATCAGGGT CAAAGAGGGG CTGCCATCTG 240

GATTCCCATG TACTTCCCAG GTGAACAGCA TAAATCACT 279

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown.

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV-3/88

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CAATAGAGGA TGGCCCTTTA ATTTATGCTG AGCATGCCAA GTACAAAAAT CATTTTGATG 60

CAGATTACAC AGCATGGGAC TCTACACAAA ATAGACAAAT AATGACAGAA TCCTTTTCCA 120

TCATGTCACG CCTCACGGCC TCTCCAGAAC TAGCTGAGGT TGTAGCCCAG GACTTGCTAG 180

CACCATCCGA GATGGATGTG GGTGACTATG TTATAAGGGT CAAAGAAGGC CTACCATCAG 240

GATTTCCCTG CACTTCTCAA GTAAATAGCA TAAATCACT 279

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/KY89

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CAATAGAGGA TGGCCCTTTA ATTTATGCTG AACATGCCAA GTACAAAAAT CATTTTGATG 60

CAGATTACAC AGCATGGGAC TCTACACAAA ATAGACAAAT TATGACAGAA TCCTTCTCCA 120

TCATGTCACG CCTTACGGCC TCTCCAGAAC TAGCTGAGGT TGTAGCCCAG GACTTACTAG 180

CACCATCCGA GATGGATGTG GGCGACTATG TTATAAGGGT CAAAGAAGGC CTACCATCAG 240

GATTTCCCTG CACTTCTCAA GTGAATAGCA TAAATCACT 279

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SRSV/Cambridge, UK/92

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

TGTATGAAGA TGGTACCATA ATATTTGAGA AACATTCCAG ATACAGATAC CACTATGATG 60

CAGATTATCC CGCTGGGTAC TCCACGCAGC AACGGGCAGT GTTGGCAGCA GCACTTGAAA 120

TCATGGTGAG GTTCTCTGCT GAACCACAGC TAGCGCAAAT AGTAGCTGAA GATCTGCTAG 180

CACCAAGTGT AGTTGATGTG GGTGACTTCA AGATCACCAT TAATGAAGGC CTACCTTCTG 240

GTGTGCCCTG CACCTCACAG TGGAACTCCA TTGCCCACT 279

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 277 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Snow Mountain Agent/78

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GAATGAGGAT GGACCCATAA TTTTTGAAAA GCACTCCAGG TTCTCATACC ACTATGATGC 60

AGATTACTCA CGCTGGGACT CAACCCAACA GAGGGCAGTG CTAGCTGCAG CCTTGGAAAT 120

CATGGTAAAA TTCTCACCAG AACCACATTT GGCCCAAATT GTTGCAGAGG ATCTCCTAGC 180

CCCCAGTGTG ATGGATGTAG GTGATTTCAA AATAACAATT AATGAGGGAC TGCCCTCGGG 240

AGTACCCTGC ACATCACAGT GGAATTCCAT GCCCACT 277

CLAIMS

1. A cDNA sequence of the formula shown in Table 2 and fragments and derivatives thereof having sufficient size to bind a Norwalk or Norwalk-related virus genome.

2. A protein encoded by nucleotides including nucleotides 1 through 7753 of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

3. The protein of claim 2, wherein said protein is produced in a prokaryotic expression system or a eukaryotic expression system.

4. The protein of claim 2, wherein said protein is produced by chemical methods.

5. A protein encoded by nucleotides 146 through 5359 of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

6. The protein of claim 5, wherein said protein is produced in a prokaryotic expression system or eukaryotic expression system.

7. The protein of claim 5, wherein said protein is produced by chemical methods.

8. A RNA-dependent RNA polymerase encoded by nucleotides 4543 to 4924 of the Norwalk virus genome shown in Table 2 or fragments.

9. The RNA polymerase of claim 8, wherein said RNA polymerase is produced in a prokaryotic expression system or a eukaryotic expression system.

10. The RNA polymerase of claim 8, wherein said RNA polymerase is produced by chemical methods.

11. A protein encoded by nucleotides 5337 through 7573 of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

12. The protein of claim 11, wherein said protein is produced in a prokaryotic expression system or eukaryotic expression system.

13. The protein of claim 11, wherein said protein is produced by chemical methods.

14. A protein encoded by nucleotides 5346 through 6935 of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

15. The protein of claim 14, wherein said protein is produced in a prokaryotic expression system or eukaryotic expression system.

16. The protein of claim 14, wherein said protein is produced by chemical methods.

17. A protein encoded by nucleotides 6938 through 7573 of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

18. The protein of claim 17, wherein said protein is produced in a prokaryotic expression system or eukaryotic expression system.

19. The protein of claim 17, wherein said protein is produced by chemical methods.

20. A method of making a RNA probe to detect Norwalk or Norwalk-related viruses, comprising the steps of:

subcloning a Norwalk virus cDNA clone into a transcription vector;

growing said cDNA containing transcription vector;

adding RNA polymerase to generate single stranded RNA by in vitro transcription; and

isolating said single stranded RNA.

21. A method of identifying Norwalk or Norwalk-related viruses in a sample suspected of containing Norwalk or Norwalk-related viruses, comprising the steps of:

adding a cDNA or a RNA probe specific to Norwalk virus or a Norwalk-related virus to said sample to be tested under conditions in which the cDNA or RNA probe will bind to the Norwalk or Norwalk-related virus genome; and

measuring the amount of binding of said cDNA or RNA probe.

22. The method of claim 21, wherein said sample is selected from the group consisting of food, water and stool.

23. The method of claim 21, wherein said cDNA is selected from a group consisting of pUCNV-953, pUCNV-4145, pUCNV-4095, pUCNV-5030 and pUCNV-5101 or fragments or derivatives thereof.

24. A method of identifying Norwalk or Norwalk-related viruses in a sample suspected of containing Norwalk or Norwalk-related viruses comprising the steps of:

adding at least two oligonucleotides each of about 10 nucleotides or greater to said sample under conditions in which said oligonucleotides bind to the Norwalk or Norwalk-related virus genome;

amplifying a nucleotide sequence between said bound oligonucleotides; and

measuring the amount of amplified sequence.

25. A method of identifying Norwalk or Norwalk-related viruses in a sample suspected of containing Norwalk or Norwalk-related viruses comprising the steps of:

isolating said nucleic acids using CTAB procedure;

amplifying nucleic acid; and

measuring the amplified product.

26. The method of claim 25, wherein the CTAB procedure includes:

extracting said sample with genetron;

removing the supernatant of said genetron extracted sampled;

precipitating viruses in said supernatant with polyethylene glycol;

treating said precipitate with proteinase K in the presence of SDS at about 30° minutes;

sequentially extracting said treated precipitate with phenol-chloroform and then chloroform;

forming a mixture by adding a solution of about 5% CTAB and about 0.4M NaC1 to said supernatant of said sequentially extracted sample at a ratio of about 5:2 sample:CTAB;

incubating said mixture;

centrifuging said mixture to collect nucleic acids;

suspending said nucleic acids in 1M NaCL and thereafter extracting with chloroform.

27. A method of claim 25 further comprising:

performing reverse transcription on said nucleic acids;

amplifying nucleic acids using primers; and

detecting the amplified nucleic acids using agarose gel electrophoresis.

28. A method of cloning Norwalk or pathogens from food, biological and environmental samples, comprising:

isolating said nucleic acids using CTAB procedure;
amplifying nucleic acids; and
incorporating said amplified nucleic acids into vectors.

29. A primer sequence of the formula CTT GTT GGT TTG AGG CCA TAT.

30. A primer sequence of the formula ATA AAA GTT GGC ATG AAC A.

31. A primer sequence of the formula GTT GAC ACA ATC TCA TCA TC.

32. A primer sequence of the formula GGC CTG CCA TCT GGA TTG CC.

33. A primer sequence of the formula GGG CCC CCT GGT ATA GGT AA.

34. A primer sequence of the formula TGG TGA TGA CTA TAG CAT CAG ACA CAA A.

35. A primer sequence of the formula ACT CAC CCA AAT CCT CCA.

36. A primer sequence of the formula GTT CTG ACC ACC TAA CCT.

37. A primer sequence of the formula AGT TTG GGT CCC CAT CTT AAT CCT TT.

38. A primer sequence of the formula TGA ACC AAA ACC AGG GGG.

39. A primer sequence of the formula AGC AAA GTC ATA CAT GAA AT.

40. A primer sequence of the formula CCA TTA TAC ATT TGT AG.

41. A primer sequence of the formula ATT ATA GTT TCT TGC ATA.

42. A primer sequence of the formula CAC ACT CTG GAC ATT GTC TG.

43. A primer sequence of the formula CAT TGG GTT TCC AGA CCT A.

44. A primer sequence of the formula ATA ATT GGG GAT CTT CCA AA.

45. A primer sequence of the formula TAG TGG CAT GGG TAT TTC.

46. A primer sequence of the formula TAT GCC AAT CAC AGC CAC.

47. A primer sequence of the formula GTC TGG CTC CCA AGT TGA CC.

48. A primer sequence of the formula CGG TAT CAG GGT CAA CAT.

49. A primer sequence of the formula TGA GGC TGC CCT GCT CCA.

50. A primer sequence of the formula CCA CCG CTG TCC GGG AGG.

51. A primer sequence of the formula GTT GCT GTT GGC ATT AAC A.

52. A method of making a probe to detect Norwalk or Norwalk-related viruses, comprising the steps of:

synthesizing one or more short or long nucleotides from the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

53. The probe produced by the method of claim 52.

54. A method of making a probe to detect Norwalk or Norwalk-related viruses, comprising the step of:

synthesizing one or more short or long nucleotides from a subgenomic region of the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

55. The probe produced by the method of claim 54.

56. The probe of claim 55, wherein said subgenomic region includes a sequence of the formula CTT GTT GGT TTG AGG CCA TAT.

57. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula ATA AAA GTT GGC ATG AAC A.

58. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula GTT GAC ACA ATC TCA TCA TC.

59. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula GGC CTG CCA TCT GGA TTG CC.

60. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula GGG CCC CCT GGT ATA GGT AA.

61. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula TGG TGA TGA CTA TAG CAT CAG ACA CAA A.

62. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula GTT CTG ACC ACC TAA CCT.

63. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula AGT TTG GGT CCC CAT CTT AAT CCT TT.

64. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula TGA ACC AAA ACC AGG GGG.

65. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula AGC AAA GTC ATA CAT GAA AT.

66. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula CCA TTA TAC ATT TGT AG.

67. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula CAC ACT CTG GAC ATT GTC TG.

68. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula CAT TGG GTT TCC AGA CCT A.

69. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula ATA ATT GGG GAT CTT CCA AA.

70. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula TAT GCC AAT CAC AGC CAC.

71. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula GTC TGG CTC CCA AGT TGA CC.

72. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula CGG TAT CAG GGT CAA CAT.

73. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula TGA GGC TGC CCT GCT CCA.

74. The probe of claim 55, wherein said subgenomic region includes a nucleotide sequence of the formula CCA CCG CTG TCC GGG AGG.

75. The method of claim 54, wherein said subgenomic region includes said Norwalk genome's first open reading frame.

76. The probe produced by the method of claim 75.

77. The method of claim 54, wherein said subgenomic region includes nucleotides 146 through 5359.

78. The probe produced by the method of claim 77.

79. The method of claim 54, wherein said nucleotides code for a picornavirus 2C-like protein, a 3C-like protease, an RNA-dependent RNA polymerase or any combination thereof.

80. The probe produced by the method of claim 79.

81. The method of claim 54, wherein said nucleotide codes for a capsid protein.

82. The probe produced by the method of claim 81.

83. The method of claim 54, wherein said subgenomic region includes nucleotides 5337 through 7573.

84. The probe produced by the method of claim 83.

85. The method of claim 54, wherein said subgenomic region includes nucleotides 5346 through 6935.

86. The probe produced by the method of claim 85.

87. The method of claim 54, wherein said subgenomic region includes nucleotides 6938 through 7573.

88. The probe produced by the method of claim 87.

89. A method of making a probe to detect Norwalk-related viruses, comprising the steps of:

selecting one or more nucleotide sequences from the group consisting of GTTGCTGTTGGCATTAACA, TAGTGGCATGGGTATTTC, ATTATAGTTTCTTGCATA, AGCAAAGTCATACATGAAAT, and ACTCACCCAAATCCTCCA;

producing said nucleotide sequence by chemical methods or in an expression system.

90. The probe produced by the method of claim 89.

91. A kit for detecting an immune response to Norwalk virus, comprising:

a container including a protein encoded by the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

92. The kit of claim 91, wherein said protein is selected from the group consisting of the protein encoded by nucleotides 1 through 7753, the protein encoded by nucleotides 146 through 5359, the protein encoded by nucleotides 5337 through 7573, the protein encoded by nucleotides 5346 through 6935, the protein encoded by nucleotides 6938 through 7573 and any combination thereof.

93. A kit for detecting an immune response to a Norwalk-related virus, comprising:

a container including a protein encoded by the genome for said Norwalk-related virus.

94. A method of detecting an immune response to Norwalk virus, comprising the steps of:

collecting a serum sample from an individual suspected of having been exposed to Norwalk virus;

selecting a protein encoded by the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof;

adding said selected protein to said serum in a diagnostic assay under conditions allowing said selected protein and the serum to react; and

measuring the amount of reaction of said serum and said selected protein.

95. The method of claim 94, wherein said diagnostic assay is selected from the group consisting of enzyme-linked immunosorbent assays, radioimmunoassays and immunoblots.

96. The method of claim 94, wherein said selected protein is a capsid protein.

97. The method of claim 94, wherein said selected protein has the intrinsic property of being able to form particle(s).

98. The method of claim 94, wherein said selected protein is selected from the group consisting of the protein encoded by nucleotides 1 through 7753, the protein encoded by nucleotides 146 through 5359, the protein encoded by nucleotides 5337 through 7573, the protein encoded by

nucleotides 5346 through 6935, the protein encoded by nucleotides 6938 through 7573 and any combination thereof.

99. A diagnostic assay to detect an immune response to Norwalk virus, comprising:

selecting a protein encoded in Norwalk virus genome shown in Table 2 or fragments or derivatives thereof;

using said protein as an antigen;

adding post-infection serum from a Norwalk infected individual under conditions allowing said serum to react with said antigen; and

measuring the amount of reaction of said serum and said antigen.

100. The method of claim 99, wherein said protein is a capsid protein.

101. The method of claim 99, wherein said protein has the intrinsic property of being able to form particle(s).

102. The method of claim 99, selected from the group consisting of the protein encoded by nucleotides 1 through 7753, the protein encoded by nucleotides 146 through 5359, the protein encoded by nucleotides 5337 through 7573, the protein encoded by nucleotides 5346 through 6935, the protein encoded by nucleotides 6938 through 7573 and any combination thereof.

103. A kit for detecting Norwalk viruses and Norwalk-related viruses, comprising:

a container including at least one antiserum made from a protein encoded by the Norwalk virus genome shown in Table 2 or from a fragment or derivative of said genome.

104. The kit of claim 102, wherein said protein is selected from the group consisting of the protein encoded by nucleotides 1 through 7753, the protein encoded by nucleotides 146 through 5359, the protein encoded by nucleotides 5337 through 7573, the protein encoded by nucleotides 5346 through 6935, the protein encoded by nucleotides 6938 through 7573 and any combination thereof.

105. A method of producing antibodies to Norwalk and Norwalk-related viruses, comprising:

immunizing animals with a protein encoded by the Norwalk virus genome shown in Table 2 or fragments or derivatives thereof.

106. The method of claim 105, wherein said protein is selected from the group consisting of the protein encoded by nucleotides 1 through 7753, the protein encoded by nucleotides 146 through 5359, the protein encoded by nucleotides 5337 through 7573, the protein encoded by nucleotides 5346 through 6935, the protein encoded by nucleotides 6938 through 7573 and any combination thereof.

107. A vaccine for Norwalk virus, comprising:

a Norwalk virus antigen encoded by the cDNA sequence of Norwalk virus shown in Table 2 or fragments or derivatives thereof.

108. The vaccine of claim 107, wherein said antigen is produced using nucleotides 146 through 5359 of the Norwalk virus genome shown in Table 2 or a derivative thereof.

109. The vaccine of claim 107, wherein said antigen is produced using nucleotides 5337 through 7573 of the Norwalk virus genome shown in Table 2 or a derivative thereof.

110. The vaccine of claim 107, wherein said antigen is produced using nucleotides 5346 through 6935 of the Norwalk virus genome shown in Table 2 or a derivative thereof.

111. The vaccine of claim 107, wherein said antigen is produced using nucleotides 6938 through 7573 of the Norwalk virus genome shown in Table 2 or a derivative thereof.

112. The vaccine of claim 107, wherein said antigen has the intrinsic property of being able to form particle(s).

113. A method of immunizing an individual against Norwalk virus, comprising the step of:

orally or parenterally administering an immunologically effective dose(s) of the vaccine of claim 107.

114. A method of immunizing an individual against Norwalk virus, comprising the steps of:

orally and parenterally administering an immunologically effective dose of the vaccine of claim 107.

115. A cDNA sequence of the human calicivirus Sopporo genome shown in Figure 9 and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

116. A protein encoded by nucleotides including nucleotides 1 through 551 of the human calicivirus Sopporo genome shown in Figure 9 or fragments or derivatives thereof.

117. A cDNA subclone of the human calicivirus Sopporo genome comprising nucleotides 1 through 149 and fragments and derivatives

thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

118. A cDNA subclone of the human calcicivirus Sopporo genome comprising nucleotides 113 through 551 and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

119. A cDNA sequence of the Day care calicivirus genome shown in Figure 9 and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

120. A cDNA sequence of the SRSV/KY/89 genome shown in Figure 12 and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

121. A cDNA sequence of the human calicivirus Houston shown in Table 10 and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

122. A cDNA subclone of a primate calicivirus comprising the sequence TGGACGGACC TGCTGTTGAA GATCTCTTCA AANGGCTCGA ACGACCAAAG CACGATCGGT ATTGTGTTGA CTACGCAAAG TGGGACTCAA CCCANCCACCA AAAGTAACAT CCAATCAATN GACATC and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

123. A cDNA subclone of a primate calicivirus comprising the sequence GTGANATGNN ACATCTTCGA CTCGATGGAC CTATTCACAT

ATGGTGATGA CGGTGTCTAC ATCGTCCCAC CACTATATCA TCTGTCATGC CCAAGTCTTC ACCAACCTGA AAC and fragments and derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

124. A method of detecting an immune response to Norwalk or a Norwalk related virus, comprising the steps of:

collecting a serum sample from an individual suspected of having been exposed to Norwalk or a Norwalk related virus;

selecting a protein encoded by the genomic sequence of a Norwalk-related virus or fragments or derivatives thereof, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome;

adding said selected protein to said serum in a diagnostic assay under conditions allowing the selected protein and the serum to react; and

measuring the amount of reaction of said serum and said selected protein.

125. The method of claim 124, wherein said diagnostic assay is selected from the group consisting of enzyme-linked immunosorbent assays, radioimmunoassays and immunoblots.

126. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 117.

127. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 119.

128. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 120.

129. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 121.

130. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 122.

131. The method of claim 124, wherein said genomic sequence is the cDNA sequence of claim 123.

132. A kit for detecting Norwalk viruses and Norwalk-related viruses, comprising:

a container including at least one antiserum made from a protein encoded by genomic sequence of a Norwalk-related virus genome or from a fragment or derivative said genomic sequence, said fragments and derivatives having sufficient size and nucleotide homology to bind a Norwalk or Norwalk-related virus genome.

133. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 117.

134. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 119.

135. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 120.

136. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 121.

137. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 122.

138. The kit of claim 132, wherein said genomic sequence is the cDNA sequence of claim 123.

139. A chimeric protein, comprising:

a protein encoded by a Norwalk virus genome combined with a protein encoded by a genome of a Norwalk-related virus.

140. A method of detecting an immune response to Norwalk virus, comprising the steps of:

collecting a serum sample from an individual suspected of having been exposed to Norwalk virus;

adding said the chimeric protein of claim 139 to said serum in a diagnostic assay under conditions allowing chimeric protein and the serum to react; and

measuring the amount of reaction of said serum and said chimeric protein.

141. A vaccine for Norwalk or Norwalk related viruses, comprising

the chimeric protein of claim 139 used as an antigen.

142. A kit for detecting Norwalk or Norwalk-related related viruses, comprising:

a container including at least one antiserum made from the chimeric protein of claim 139.

Figure la

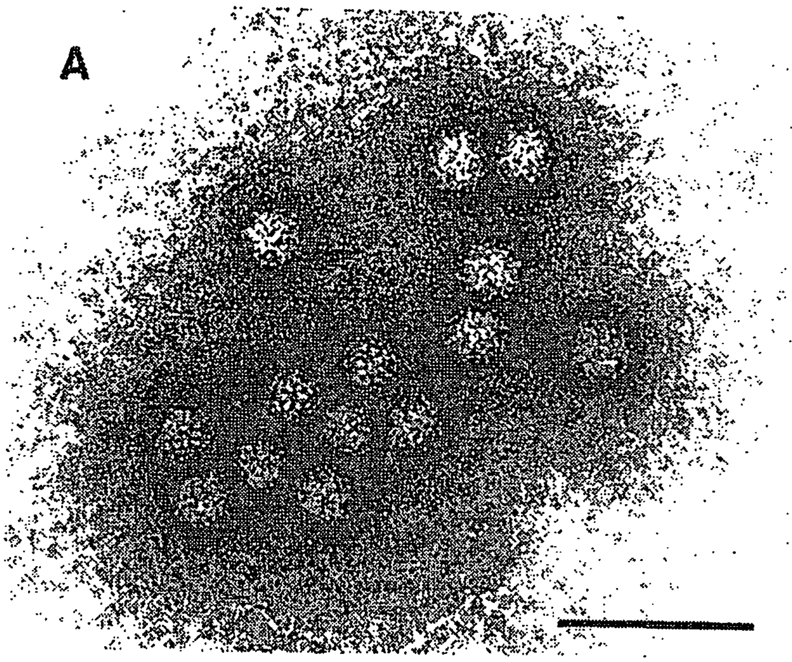


Figure 1b

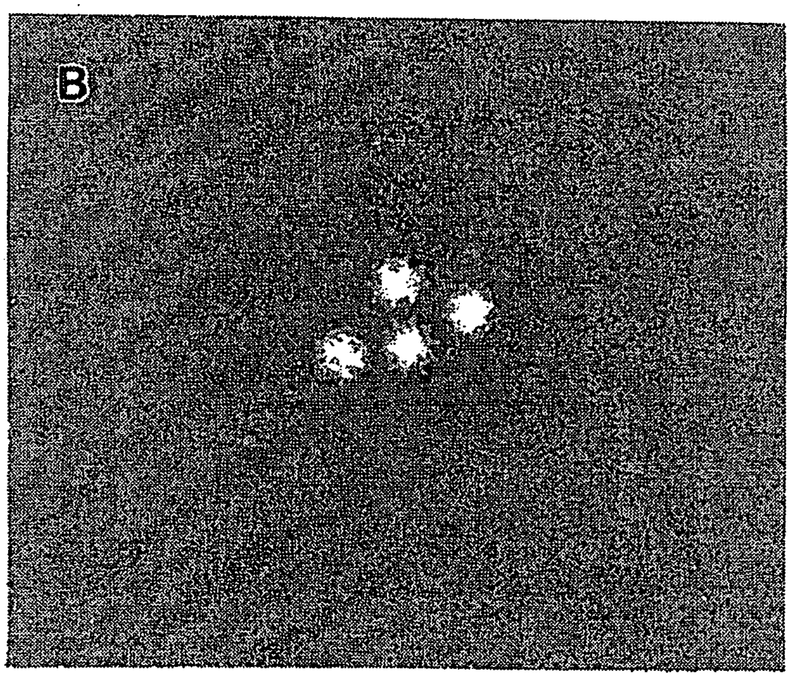


Figure 1c

Figure 1d

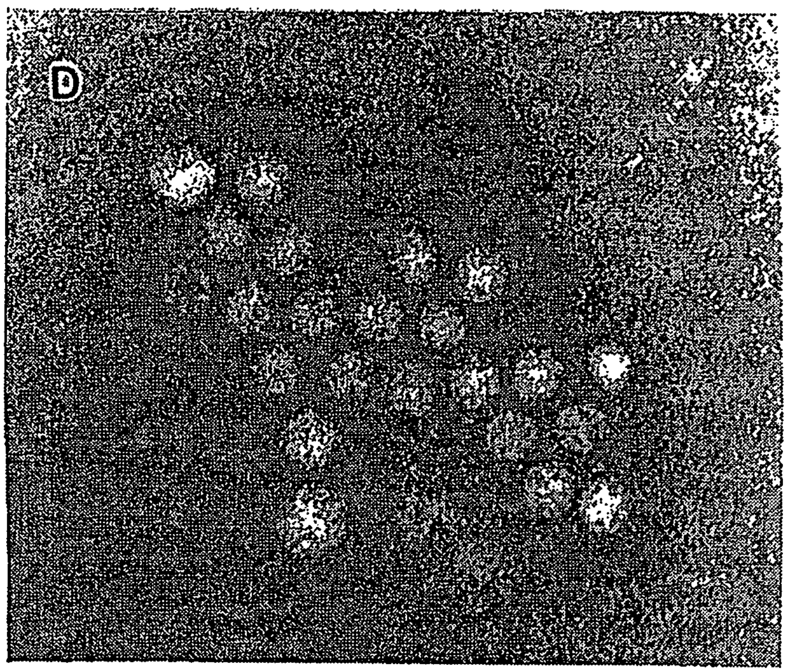


Figure 2a

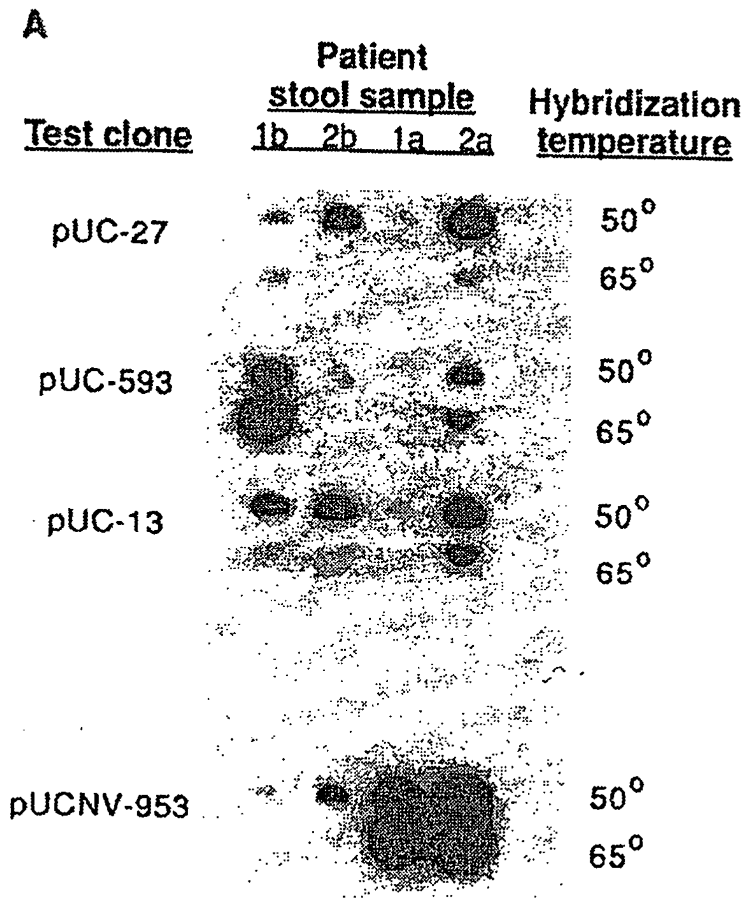
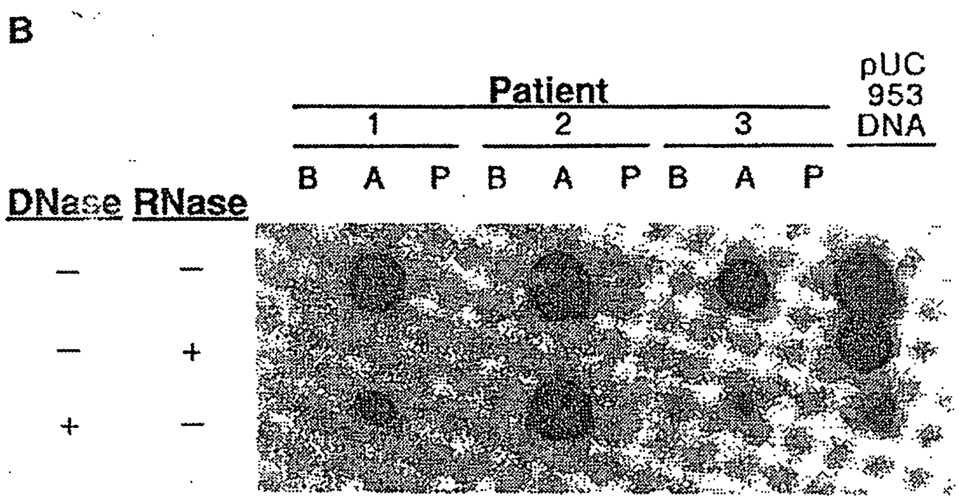

A
Patient
stool sample
Test clone
1b 2b 1a 2a
Hybridization
temperature
pUC-27
50°
65°
pUC-593
50°
65°
pUC-13
50°
65°
pUCNV-953
50°
65°

Figure 2b



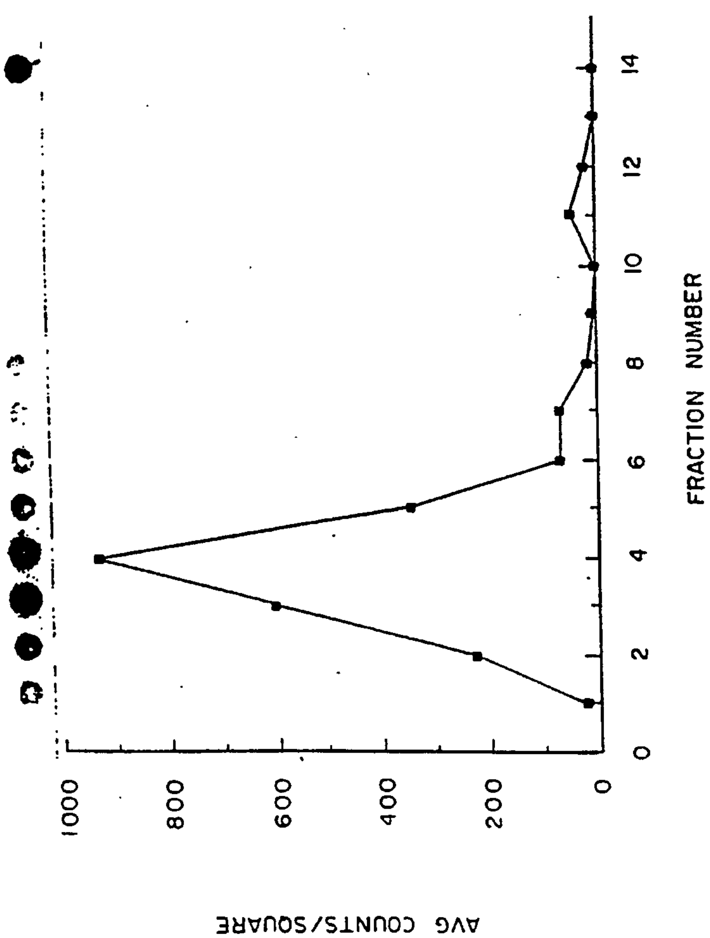

AVG COUNTS/SQUARE
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
FRACTION NUMBER

FIG. 3

```
                                    21                                         41
G TGC TCT GGG AGC GGG CAT ACA GGT TGG TGG CGA CAG GCC CTC CAA
  cys ser gly ser gly his thr gly trp trp arg gln ala leu gln

                        61                                  81
  AGC CAA AGG TAT CAA CAA AAT TTG CAA CTG CAA GAA AAT TCT TTT
  ser gln arg tyr gln gln asn leu gln leu gln glu asn ser phe

              101                                 121
  AAA CAT GAC AGG GAA ATG ATT GGG TAT CAG GTT GAA GCT TCA AAT
  lys his asp arg glu met ile gly tyr gln val glu ala ser asn

      141                                 161                      18
  CAA TTA TTG GCT AAA AAT TTG GCA ACT AGA TAT TCA CTC CTC CGT
  gln leu leu ala lys asn leu ala thr arg tyr ser leu leu arg

  1                           201                              221
  GCT GGG GGT TTG ACC AGT GCT GAT GCA GCA AGA TCT GTG GCA GGA
  ala gly gly leu thr ser ala asp ala ala arg ser val ala gly

                      241                             261
  GCT CCA GTC ACC CGC ATT GTA GAT TGG AAT GGC GTG AGA GTG TCT
  ala pro val thr arg ile val asp trp asn gly val arg val ser

              281                         301
  GCT CCC GAG TCC TCT GCT ACC ACA TTG AGA TCC GGT GGC TTC ATG
  ala pro glu ser ser ala thr thr leu arg ser gly gly phe met

      321                             341                          36
  TCA GTT CCC ATA CCA TTT GCC TCT AAG CAA AAA CAG GTT CAA TCA
  ser val pro ile pro phe ala ser lys gln lys gln val gln ser

  1                           381                              401
  TCT GGT ATT AGT AAT CCA AAT TAT TCC CCT TCA TCC ATT TCT CGA
  ser gly ile ser asn pro asn tyr ser pro ser ser ile ser arg

                      421                             441
  ACC ACT AGT TGG GTC GAG TCA CAA AAC TCA TCG AGA TTT GGA AAT
  thr thr ser trp val glu ser gln asn ser ser arg phe gly asn

              461                             481
  CTT TCT CCA TAC CAC GCG GAG GCT CTC AAT ACA GTG TGG TTG ACT
  leu ser pro tyr his ala glu ala leu asn thr val trp leu thr

      501
  CCA CCC GGT TCA ACC
  pro pro gly ser thr
```

FIG 4

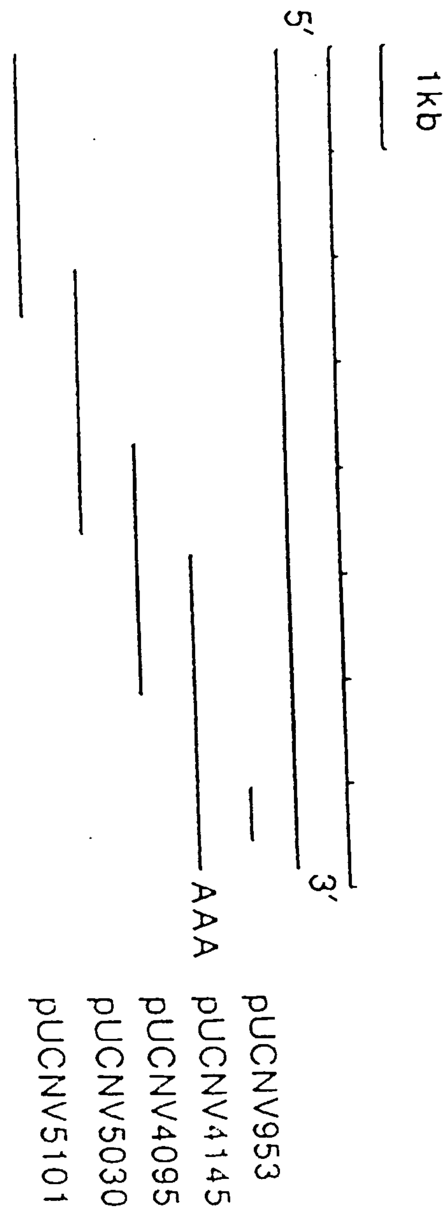

1kb
5′
3′
AAA
pUCNV953
pUCNV4145
pUCNV4095
pUCNV5030
pUCNV5101

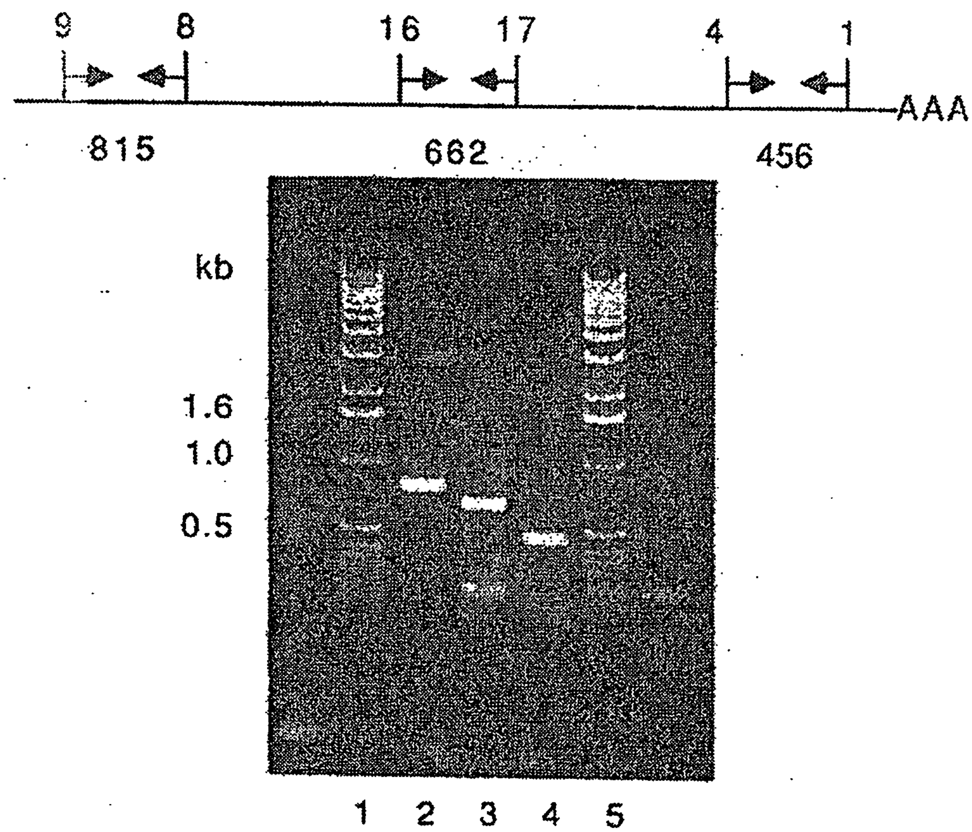
FIG 5

```
NV      HFDADYTAWDSTQNRQIMT-ESFSIMSR--LTASPEL-AEVVAQDLLAPSEMDV-----GDYVIRVK--EG-LPSGFPCTSQVN
HEV     VFENDFSEFDSTQNNFSLG-LECAIMEEC-GMPQWLI-RLYHLIRSAWILQAPKESLR-GFWKKH-----S-KHSGEPGTLLWN
HCV     GFSYDTRCFDSTVTESDIR-TEEAIYQCCDLDPQARV-AIKSLTERLYVGGPLTNSR--GENCGYRRCRAS-RASGVLTTSCGN
HAV     GLDLDFSAFDASLSPFMIREAGRIMSELS-GTPSHFGTALINTIIYSKHLLYNCCYHVCGS-----------MPSGSPCTALLN
JE      MYADDTAGWDTRITRTDLE-NEAKVLELLDGEHRMLARAIIELTYRHKVVKVMRPAAE-GKTVMDVISREDQRGSGQVVTYALN
POLIO   FA-FDYTGYDASLS-PAWFEAL-KMVLEKIGFGDRVDY-IDYLNHSHHLYKNKTYCVKGG------------MPSGCSGTSIFN
FMD     VWDVDYSAFDANHCSDAMNIMFEEVFRTDFGFHPNAEWILKTLVNTEHAYENKRITV-EGG-----------MPSGCSATSIIN
EMC     VYDVDYSNFDSTHSVAMFRLLAEEFFTPENGF-DPLTREYLESLAISTHAFEEKRFLITGG-----------LPSGCAATSMLN
SNBV    VLETDIASFDKS-QDDAMALTGLMILEDL-GVDQPLLDLIECAFGEISSTHLPTGTRFKFGA--M-------MKSGMFLTLFVN
THV     VLELDISKYDKS-QNEFHCAVEYEIWRRL-GFEDFLGEVWKQG--HRKTTLK-DITA--GYKTC--IWY--QRKSGDVTTFIGN
AMV     FKEIDFSKFDKS-QNELHHLIQERFLKYL-GIPNEFLTLWFNA--HRKSRIS-DSKN--GVFFN-VDF---QRRTGDALTYLGN
BMV     FLEADLSKFDKS-QGELHLEFQREILLAL-GFPAPLTNWWSDF--HRDSYLS-DPHAKVGMS---VSF---QRRTGDAFTYFGN
CpMV    VLCCDYSSFDGLLSKQVMDVIASMINELCGGE-DQLKNARRNLLMACCSRLAICKNTVWRVECG--------IPSGFPMTVIVN

NV      SINHWIITLCALSEATGLSPD---------------------VVQSMSYFSFYGDDEIVS----TDIDFDP-ARLTQILK--E
HEV     TVWNMAVITHC------------------------------YDFRDFQVAAFKGDDSIVL-CSEYRQSPG--A-A--VLIAGC
HCV     TLTCYIKARAACRAAGLQDCTMLVC----------------------------GDDLVVI-CESAGVQED--A-A--SL-RAF
HAV     SIINNVNLYYVFSKI---------------------FGKSPVFFCQALKILCYGDDVLIV--FSRDVQIDNLDLIGQKIVDEF
JE      TFTNIAVQLVRLMEAEGVIGPQHLEQLPRKTKIAVRTWLFENGEERVTRMAISGDDCVVK------PLDDRFATALHFL-NAM
POLIO   SMINNLIIRTLLLKTYKGID------------------------LDHLKMIAYGDDVIAS----YPHEVDAS------LLAQS
FMD     TILNNIYVLYALRRHYEGVE------------------------LDTYTMISYGDDIVVA------SDYDLDF---EALKP-H
EMC     TIMNNIIIRAGLYLTYKNFE------------------------FDDVKVLSYGDDLLVA--TNYQLDFDKV---RASLAKTG
SNBV    TVLNVVIASRVLE----------------------------ERLKTSRCAAFIGDDNIIH-----GVVSDKEMAERCATWL-N
THV     TVIIAACLASML------------------------------PMEKIIKGAFCGDDSLLY-FPKGCEFPDVQHSAN-LMWNFE
AMV     TIVTLACLCHVYDLM----------------------------DPNVKFVVASGDDSLIG--TVEELPRDQEF-LFTTLFNLE
BMV     TLVTHAMIAYASDLS------------------------------DCDCAIFSGDDSLII--SKVKPVLDTDM--FTSLFNME
CpMV    SIFNEILIRYHYKKLMREQQAPE---------------LMVQSFDKLIGLVTYGDDNLISVNAVVTPYFDGKKL-KQSLAQGG
```

FIG 6

Figure 7



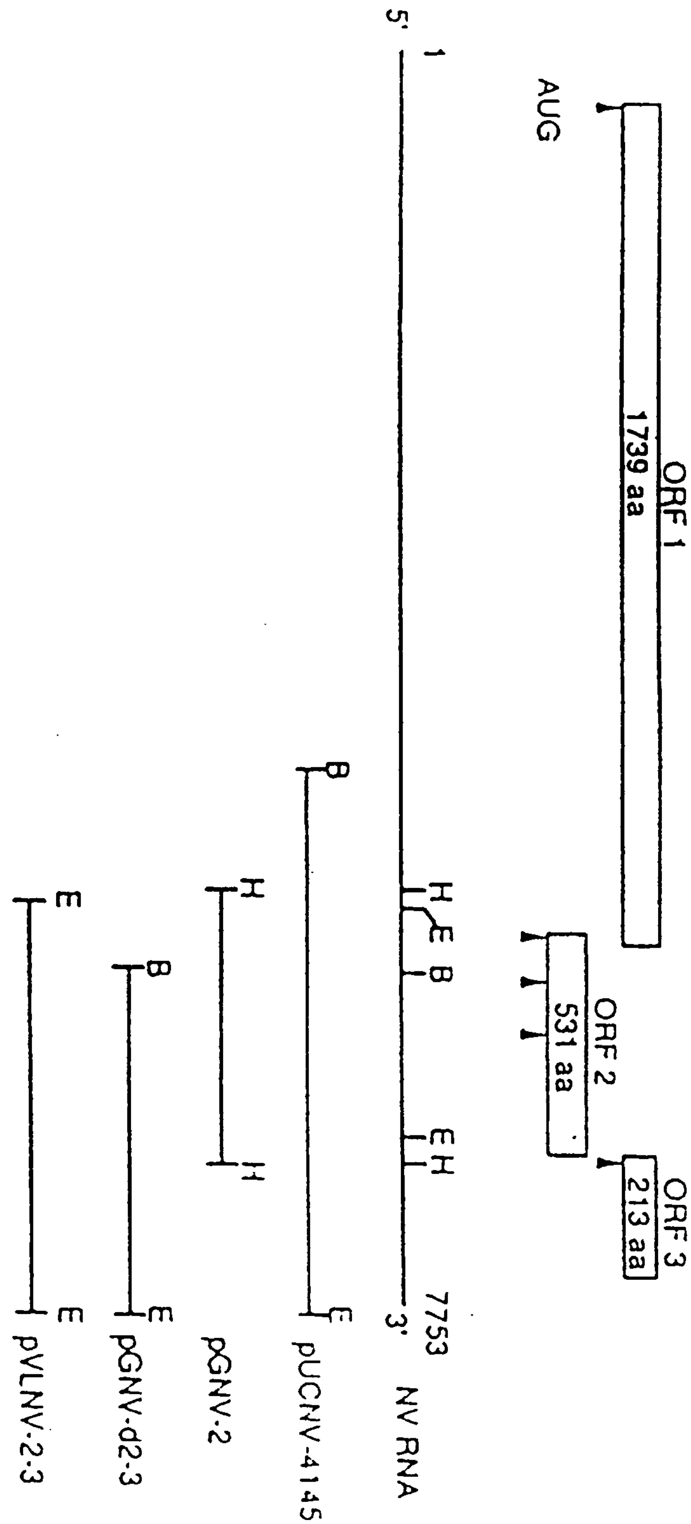

5'
1
AUG
ORF 1
1739 aa
ORF 2
531 aa
ORF 3
213 aa
7753
3'
NV RNA
pUCNV-4145
pGNV-2
pGNV-d2-3
pVLNV-2-3

FIG 8

Figure 9(1)

```
                                                                      45
TGT GAT GCT GCC ACC ACG CTT ATA GCC ACC GCG GCT TTT AAG GCC   HuCV Sapporo
 C   D   A   A   T   T   L   I   A   T   A   A   F   K   A    Sapporo a.a.
←.........at23s2m31..............................................

                                                                      90
GTG GCT ACN AGG CTA CAG GTG GTG ACA CCA ATG ACA CCA GTT GCT   HuCV Sapporo
 A   V   X   R   L   Q   V   V   T   P   M   T   P   V   A    Sapporo a.a.
...............................................................
                                                        ⇦.......

                                                                     135
                ... ... ... ... ... ... ... ... ... ... ...   Day care
GTT GGC ATT AAC ATG GAC TCT GTT CAG ATG CAA GTG ATG AAT GAC   HuCV Sapporo
 V   G   I   N   M   D   S   V   Q   M   Q   V   M   N   D    Sapporo a.a.
...............................................................
.....primer new 36.......⇨
            ←..............................................c-29_4-gcl..........

                                                                     180
... ... ... ..A ... ... ..C ... ..C C.. ... ..C ... ... ...   Day care
TCT TTA AAG GGG GGT GTT CTT TAC TGT TTG GAT TAT TCC AAA TGG   HuCV Sapporo
 S   L   K   G   G   V   L   Y   C   L   D   Y   S   K   W    Sapporo a.a.
..................→
...............................................................
```

Figure 9(2)

```
                                                              225
..C ... ... ... ..T G.. ... ... ... ... ..A ... ..N N.. ...   Day care
GAT TCC ACA CAA AAC CCT GCA GTG ACA GCA GCC TCC CTG GCA ATA   HuCV Sapporo
 D   S   T   Q   N   P   A   V   T   A   A   S   L   A   I    Sapporo a.a.
.............................................................

                                                              270
... ... ... ... ... ..A ... ..C ... ... ... ... ... ... ...   Day care
TTG GAG AGA TTT GCT GAG CCC CAT CCA ATT GTG TCT TGT GCC ATT   HuCV Sapporo
 L   E   R   F   A   E   P   H   P   I   V   S   C   A   I    Sapporo a.a.
.............................................................

                                                              315
... ..C ..G NN. ..N N.. ... ... ..T ..C ..T ... ... ... ..G   Day care
GAG GCT CTT TCC TCC CCT GCA GAG GGC TAT GTC AAT GAT ATC AAA   HuCV Sapporo
 E   A   L   S   S   P   A   E   G   Y   V   N   D   I   K    Sapporo a.a.
.............................................................

                                                              360
... ... ... ..T ... ... ... ... .G. ... ... ... ..C ... ...   Day care
TTT GTG ACA CGC GGC GGC CTA CCA TCT GGG ATG CCA TTT ACA TCT   HuCV Sapporo
 F   V   T   R   G   G   L   P   S   G   M   P   F   T   S    Sapporo a.a.
.............................................................
```

Figure 9(3)

```
                                                        405
..T ... ... ..C ... ... ..C ..N ... ... ... ..A ..C ... ...  Day care
GTC GTC AAT TCT ATC AAC CAT ATG ATA TAC GTG GCG GCA GCC ATC  HuCV Sapporo
 V   V   N   S   I   N   H   M   I   Y   V   A   A   A   I   Sapporo a.a.
.............................................................

                                                        450
... ... ... ... ... ... ... ... ..T ... ..C ... ... ..T ...  Day care
CTG CAG GCA TAC GAA AGC CAC AAT GTC CCA TAT ACT GGA AAC GTC  HuCV Sapporo
 L   G   A   Y   E   S   H   N   V   P   Y   T   G   N   V   Sapporo a.a.
.............................................................

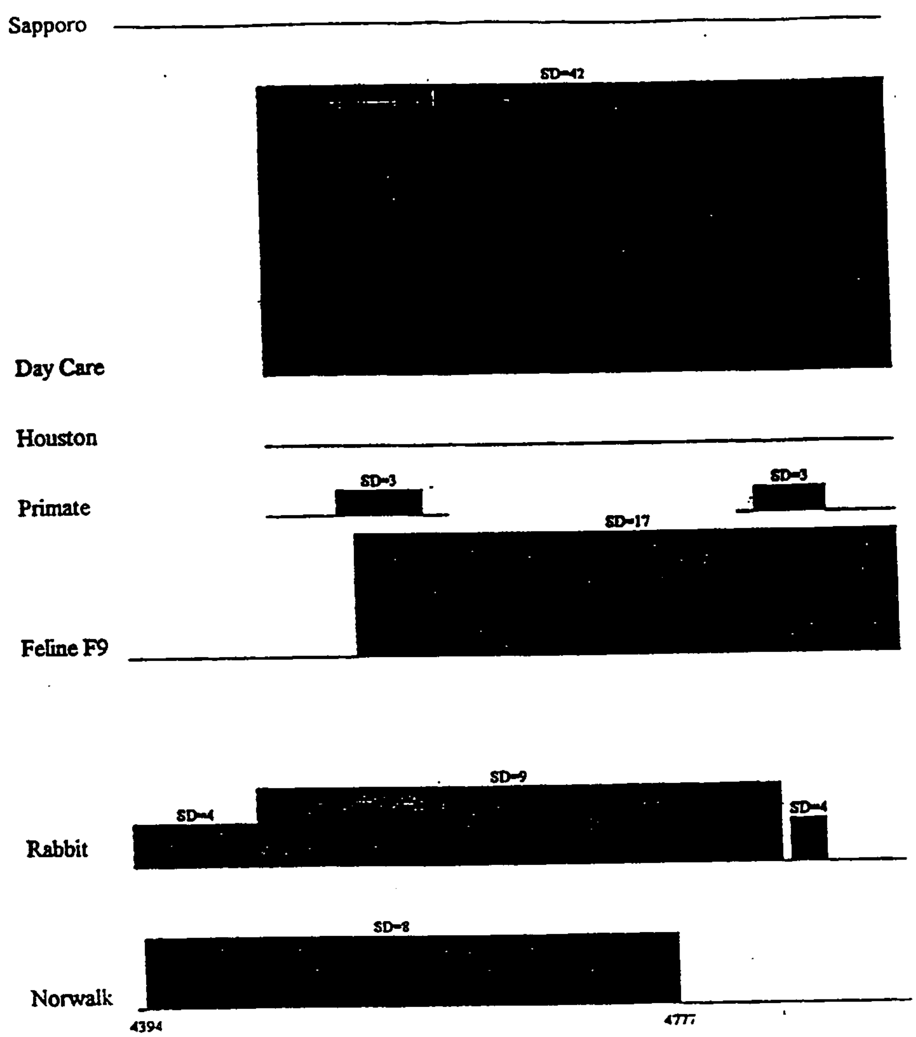
                                                        495
... ... ... ... ..T ... ... ... ... ... ..C ... ... ... ...  Day care
TTC CAA GTG GAG ACC GTT CAC ACG TAT GGT GAT GAT TGC ATG TAC  HuCV Sapporo
 F   Q   V   E   T   V   H   T   Y   G   D   D   C   M   Y   Sapporo a.a.
.............................................................
```

Figure 9(4)

```
                                                            540
...  ...  ...  ...  ...  ..C  ...  ...  ...  ...  ...  ...  ..A  ...  ...   Day care
AGC  GTG  TGC  CCT  GCC  ACT  GCA  TCA  ATT  TTC  CAC  ACT  GTG  CTT  GCA   HuCV Sapporo
 S    V    C    P    A    T    A    S    I    F    H    T    V    L    A    Sapporo a.a.
```

```
                551
...  ...  ..-  --   Day care
AAC  CTA  ACG  TC   HuCV Sapporo
 N    L    T        Sapporo a.a.
```

Figure 10


Sapporo
SD=42
Day Care
Houston
SD=3
SD=3
Primate
SD=17
Feline F9
SD=9
SD=4
SD=4
Rabbit
SD=8
Norwalk
4394
4777

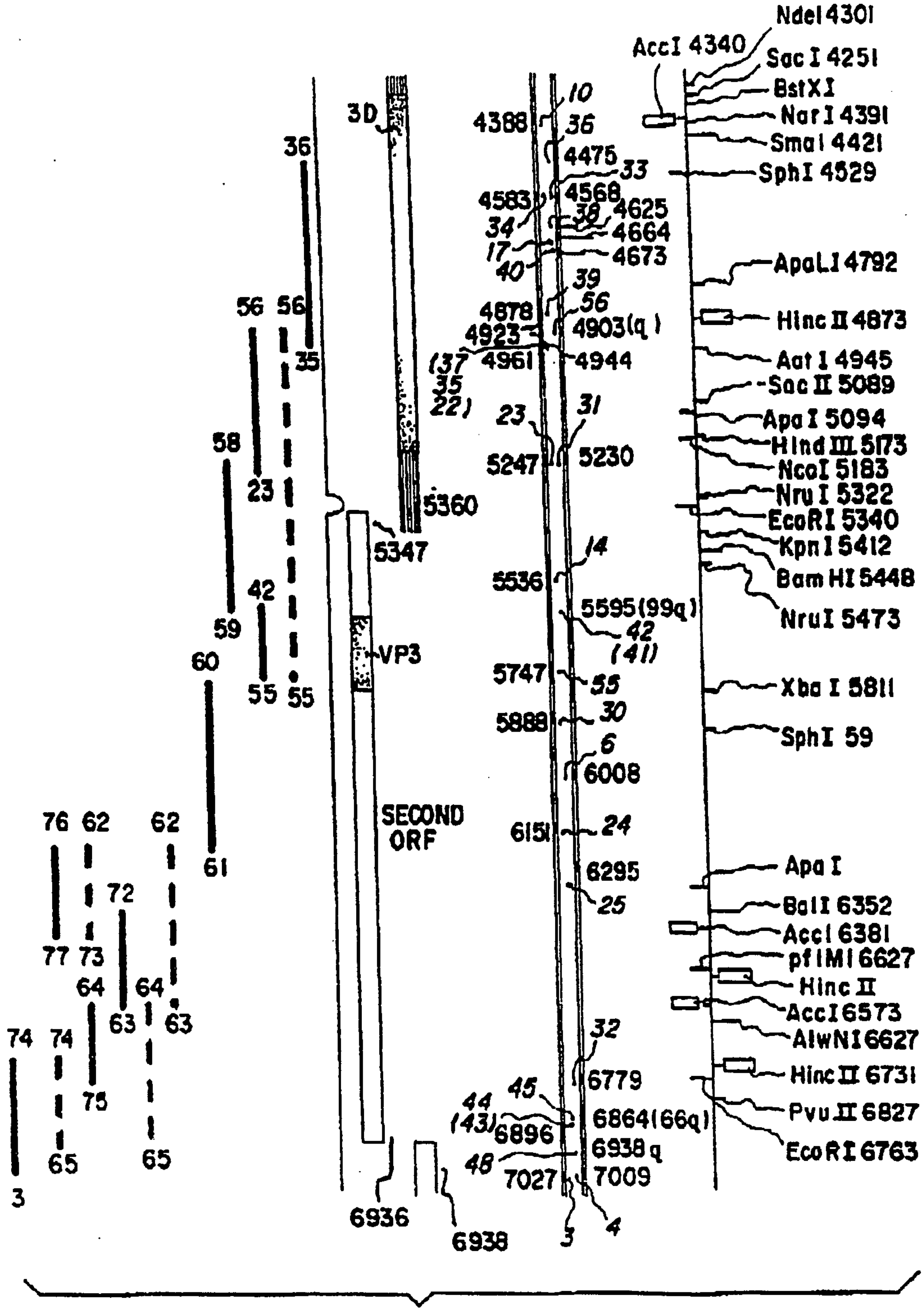

NdeI 4301
AccI 4340
SacI 4251
BstXI
NarI 4391
SmaI 4421
SphI 4529
ApaLI 4792
HincII 4873
AatI 4945
SacII 5089
ApaI 5094
HindIII 5173
NcoI 5183
NruI 5322
EcoRI 5340
KpnI 5412
BamHI 5448
NruI 5473
XbaI 5811
SphI 59
ApaI
BalI 6352
AccI 6381
pfIMI 6627
HincII
AccI 6573
AlwNI 6627
HincII 6731
PvuII 6827
EcoRI 6763
3D
VP3
SECOND ORF
5360
5347
6936
6938
4388
4475
4583
4568
4625
4664
4673
4878
4903(q)
4923
4961
4944
5247
5230
5536
5595(99q)
5747
5888
6008
6151
6295
6779
6864(66q)
6896
6938q
7027
7009

FIG. 11

Figure 12 (1)

```
4494 - CAATAGAAGA TGGCCCCCTC ATCTATGCTG AGCATGCTAA ATATAAGAAT CATTTTGATG  Norwalk virus
       ||||||| || ||||||  |  || ||||||| | ||||| ||  || || ||| ||||||||||
   1 - CAATAGAGGA TGGCCCTTTA ATTTATGCTG AACATGCCAA GTACAAAAAT CATTTTGATG  SRSV/KY/89

4554 - CAGATTATAC AGCATGGGAC TCAACACAAA ATAGACAAAT TATGACAGAA TCCTTCTCCA  Norwalk virus
       ||||||| || |||||||||| || ||||||| |||||||||| |||||||||| ||||||||||
  61 - CAGATTACAC AGCATGGGAC TCTACACAAA ATAGACAAAT TATGACAGAA TCCTTCTCCA  SRSV/KY/89

4614 - TTATGTCGCG CCTTACGGCC TCACCAGAAT TGGCCGAGGT TGTGGCCCAA GATTTGCTAG  Norwalk virus
       | ||||| || |||||||||| || ||||||  | || ||||| ||| |||||  || || ||||
 121 - TCATGTCACG CCTTACGGCC TCTCCAGAAC TAGCTGAGGT TGTAGCCCAG GACTTACTAG  SRSV/KY/89

4674 - CACCATCTGA GATGGATGTA GGTGATTATG TCATCAGGGT CAAAGAGGGG CTGCCATCTG  Norwalk virus
       ||||||| || |||||||||  || || |||| | || ||||| |||||| ||  || ||||| |
 181 - CACCATCCGA GATGGATGTG GGCGACTATG TTATAAGGGT CAAAGAAGGC CTACCATCAG  SRSV/KY/89

4734 - GATTCCCATG TACTTCCCAG GTGAACAGCA TAAATCACTG GATAATTACT CTCTGTGCAC  Norwalk virus
       |||| || ||  ||||| ||  ||||| |||| |||||||||| |||||| ||  || |||||||
 241 - GATTTCCCTG CACTTCTCAA GTGAATAGCA TAAATCACTG GATAATCACC CTTTGTGCAT  SRSV/KY/89

4794 - TGTCTGAGGC CACTGGTTTA TCACCTGATG TGGTGCAATC CATGTCATAT TTCTCATTTT  Norwalk virus
       ||||||||||  ||||| ||| |||||||||| |||| || || |||||||||  |||||||| |
 301 - TGTCTGAGGC TACTGGCTTA TCACCTGATG TGGTACAGTC CATGTCATAC TTCTCATTCT  SRSV/KY/89
                                                                     *

4854 - ATGGTGATGA TGAGATTGTG TCAACTGACA TAGATTTTGA CCCAGCCCGC CTCACTCAAA  Norwalk virus
       | |||||||| |||||| ||  |||||||||| |||| ||||| |||| | ||| ||||| ||||
 361 - ACGGTGATGA TGAGATCGTA TCAACTGACA TAGACTTTGA CCCAACTCGC CTCACCCAAA  SRSV/KY/89
       ********** ****
```

Figure 12(2)

```
4914 - TTCTCAAGGA ATATGGCCTC AAACCAACAA GGCCTGACAA AACAGAAGGA CCAATACAAG  Norwalk virus
       |||||||||| ||| |||||| || ||||||| |||| ||||| |||||||||| |||||||| |
 421 - TTCTCAAGGA ATACGGCCTC AAGCCAACAA GGCCAGACAA AACAGAAGGA CCAATACAGG  SRSV/KY/89
                  ++++++++++ ++++++++++

4974 - TGAGGAAAAA TGTGGATGGA CTGGTCTTCT TGCGGCGCAC CATTTCCCGT GATGCGGCAG  Norwalk virus
       ||||||| || |||||||||  || || ||   |||||||||| ||| |||||  || || ||||
 481 - TGAGGAAGAA TGTGGATGGG CTAGTTTTTC TGCGGCGCAC CATCTCCCGG GACGCAGCAG  SRSV/KY/89

5034 - GGTTCCAAGG CAGGTTAGAT AGGGCTTCGA TTGAACGCCA AATCTTCTGG ACCCGCGGGC  Norwalk virus
       ||||||||||  ||  | ||| || || || | ||||||| || ||| |||||| ||||||||||
 541 - GGTTCCAAGG TAGACTGGAT AGAGCCTCAA TTGAACGTCA AATTTTCTGG ACCCGCGGGC  SRSV/KY/89

5094 - CCAATCATTC AGATCCATCA GAGACTCTAG TGCCACACAC TCAAAGAAAA ATACAGTTGA  Norwalk virus
       |||| ||||| ||| |||||| |||||||| | | ||||||||  ||||| |||  | ||| |||
 601 - CCAACCATTC AGACCCATCA GAGACTCTGG TACCACACAC CCAAAGGAAA GTCCAGCTGA  SRSV/KY/89

5154 - TTTCACTTCT AGGGGAAGCT TCACTCCATG GTGAGAAATT TTACAGAAAG ATTTCCAGCA  Norwalk virus
       | |||||||| ||| |||||  |||||||| | | || ||||| |||||| ||  || || ||||
 661 - TCTCACTTCT AGGAGAAGCC TCACTCCACG GGGAAAAATT TTACAGGAAA ATATCTAGCA  SRSV/KY/89

5214 - AGGTCATACA TGAAATCAAG ACTGGTGGAT TGGAAATGTA TGTCCCAGGA TGGCAGGCCA  Norwalk virus
       | |||||||| |||||| ||| ||||||||   |||| ||||| |||||||||  ||||||||||
 721 - AAGTCATACA TGAAATTAAG ACTGGTGGGC TGGAGATGTA TGTCCCAGGG TGGCAGGCCA  SRSV/KY/89

5274 - TGTTCCGCTG GATGCGCTTC CATGACCTCG GATTGTGGAC AGGAGATCGC GATCTTCTGC  Norwalk virus
       |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||  |||| ||||
 781 - TGTTCCGCTG GATGCGCTTC CATGACCTCG GATTGTGGAC AGGAGATCGC AATCTCCTGC  SRSV/KY/89
```

Figure 12 (3)

```
5334 - CCGAATTCGT AAATGATGAT GGCGTCTAAG GACGCTACAT CAAGCGTGGA TGGCGCTAGT  Norwalk virus
 841 - CCGAATTCGT AAATGATGAT GGCGTCTAAG GACGCTACGT CAAGCGTGGA TGGCGCCAGT  SRSV/KY/89
                xxx

5394 - GGCGCTGGTC AGTTGGTACC GGAGGTTAAT GCTTCTGACC CTCTTGCAAT GGATCCTGTA  Norwalk virus
 901 - GCGTCGGTTC AGTTGGTACC GGAGGTTAAT GCTTCTGACC CTCTTGCAAT GGATCCTGTG  SRSV/KY/89

5454 - GCAGGTTCTT CGACAGCAGT CGCGACTGCT GGACAAGTTA ATCCTATTGA TCCCTGGATA  Norwalk virus
 961 - GCGGGTTCTT CAACAGCAGT TGCAACCGCT GGACAAGTTA ACCCTATTGA CCCTTGGATA  SRSV/KY/89

5514 - ATTAATAATT TTGTGCAAGC CCCCCAAGGT GAATTTACTA TTTCCCCAAA TAATACCCCC  Norwalk virus
1021 - ATCAATAACT TTGTGCAGGC TCCCCAAGGT GAATTTACTA TTTCTCCAAA TAATACCCCC  SRSV/KY/89

5574 - GGTGATGTTT TGTTTGATTT GAGTTTGGGT CCCCATCTTA ATCCTTTCTT GCTCCATCTA  Norwalk virus
1081 - GGTGATGTTT TGTTTGATTT GAGTCTAGGC CCTCATCTTA ATCCCTTCTT GTTACATTTG  SRSV/KY/89

5634 - TCACAAATGT ATAATGGTTG GGTTGGTAAC ATGAGAGTCA GGATTATGCT AGCTGGTAAT  Norwalk virus
1141 - TCACAAATGT ATAATGGCTG GGTTGGCAAC ATGAGAGTTA GGATTATGCT GGCTGGTAAT  SRSV/KY/89

5694 - GCCTTTACTG CGGGGAAGAT AATAGTTTCC TGCATACCCC CTGGTTTTGG TTCACATAAT  Norwalk virus
1201 - GCATTTACTG CAGGCAAAAT TATAGTTTCT TGCATACCTC CTGGCTTTGG CTCCCAACAA  SRSV/KY/89

5754 - CTTACTATAG CACAAGCAAC TCTCTTTCCA CATGTGATTG CTGATGTTAG GACTCTAGAC  Norwalk virus
1261 - CTTACTATAG CACAAGCAAC TCTCTTCCCG CATGTGATTG CTGATGTTAG GACTTTAGAC  SRSV/KY/89
```

Figure 12 (4)

```
5814 - CCCATTGAGG TGCCTTTGGA AGATGTTAGG AATGTTCTCT TTCATAATAA TGATAGAAAT Norwalk virus
1321 - CCAATTGAAG TACCCTTGGA AGATGTAAGG AATGTTCTCT TTCATAATAA TGATAGAAAT SRSV/KY/89

5874 - CAACAAACCA TGCGCCTTGT GTGCATGCTG TACACCCCCC TCCGCACTGG TGGTGGTACT Norwalk virus
1381 - CAACAAACTA TGCGCCTTGT GTGCATGCTT TATACCCCCC TCAGCACTGG TGGCGGTACA SRSV/KY/89

5934 - GGTGATTCTT TTGTAGTTGC AGGGCGAGTT ATGACTTGCC CCAGTCCTGA TTTTAATTTC Norwalk virus
1441 - GGTGATTCTT TTGTGGTTGC AGGGCGAGTC ATGACTTGTC CTAGCCCCGA CTTTAATTTC SRSV/KY/89

5994 - TTGTTTTTAG TCCCTCCTAC GGTGGAGCAG AAAACCAGGC CCTTCACACT CCCAAATCTG Norwalk virus
1501 - TTGTTCTTGG TTCCTCCCAC AGTGGAACAG AAGACTAGGC CTTTCACCCT CCCAAATTTA SRSV/KY/89

6054 - CCATTGAGTT CTCTGTCTAA CTCACGTGCC CCTCTCCCAA TCAGTAGTAT CGGCATTTCC Norwalk virus
1561 - CCGCTGAGTT CTTTGTCTAA TTCACGTGCT CCTCTTCCAA TTAGTGGCAT GGGTATTTCT SRSV/KY/89

6114 - CCAGACAATG TCCAGAGTGT GCAGTTCCAA AATGGTCGGT GTACTCTGGA TGGCCGCCTG Norwalk virus
1621 - CCAGATAATG TTCAGAGTGT GCAGTTCCAA AATGGCCGAT GTACCTTAGA TGGACGTCTT SRSV/KY/89

6174 - GTTGGCACCA CCCCAGTTTC ATTGTCACAT GTTGCCAAGA TAAGAGGGAC CTCCAATGGC Norwalk virus
1681 - GTTGGCACCA CCCCAGTTTC CCTCTCCCAT GTTGCTAAGA TAAGGGGTAC TTCTAATGGT SRSV/KY/89

6234 - ACTGTAATCA ACCTTACTGA ATTGGATGGC ACACCCTTTC ACCCTTTTGA GGGCCCTGCC Norwalk virus
1741 - ACAGTAATCA ATCTCACCGA ATTGGATGGC ACCCCCTTCC ACCCTTTTGA AGGCCCTGCC SRSV/KY/89
```

Figure 12 (5)

```
6294 - CCCATTGGGT TTCCAGACCT CGGTGGTTGT GATTGGCATA TCAATATGAC ACAGTTTGGC  Norwalk virus
       || ||||| | ||||||| ||  ||||| ||| |||||||||| | |||||||| ||| |||||
1801 - CCTATTGGTT TTCCAGATCT TGGTGGCTGT GATTGGCATA TTAATATGAC ACAATTTGGA  SRSV/KY/89

6354 - CATTCTAGCC AGACCCAGTA TGATGTAGAC ACCACCCCTG ACACTTTTGT CCCCCATCTT  Norwalk virus
       ||||| || | |||| ||||| |||||||||| |||||||| | |||| |  || ||| ||  |
1861 - CATTCCAGTC AGACTCAGTA TGATGTAGAC ACCACCCCCG ACACCTCCGT CCCTCACTTA  SRSV/KY/89

6414 - GGTTCAATTC AGGCAAATGG CATTGGCAGT GGTAATTATG TTGGTGTTCT TAGCTGGATT  Norwalk virus
       |||||||| | |||| ||||| |||||| ||| || || |||  |||||||||| ||||||| |
1921 - GGTTCAATCC AGGCGAATGG CATTGGTAGT GGCAACTATA TTGGTGTTCT TAGCTGGGTC  SRSV/KY/89

6474 - TCCCCCCCAT CACACCCGTC TGGCTCCCAA GTTGACCTTT GGAAGATCCC CAATTATGGG  Norwalk virus
       |||||||||| |||| || || |||||| ||| ||||| || | |||||||||| ||| ||||||
1981 - TCCCCCCCAT CACATCCATC TGGCTCTCAA GTTGATCTCT GGAAGATCCC CAACTATGGG  SRSV/KY/89

6534 - TCAAGTATTA CGGAGGCAAC ACATCTAGCC CCTTCTGTAT ACCCCCCTGG TTTCGGAGAG  Norwalk virus
       || ||||| | | ||||||||  ||||||||  || ||||| | |  | |||||  || ||||||
2041 - TCTAGTATCA CAGAGGCAAC CCATCTAGCT CCCTCTGTCT ATTCTCCTGG CTTTGGAGAG  SRSV/KY/89

6594 - GTATTGGTCT TTTTCATGTC AAAAATGCCA GGTCCTGGTG CTTATAATTT GCCCTGTCTA  Norwalk virus
       ||  | |||| |||||||||| ||| || ||| ||||||||||  |  || | | ||||||| ||
2101 - GTGCTAGTCT TTTTCATGTC AAAGATACCA GGTCCTGGTG GTGATAGTCT GCCCTGTTTA  SRSV/KY/89

6654 - TTACCACAAG AGTACATTTC ACATCTTGCT AGTGAACAAG CCCCTACTGT AGGTGAGGCT  Norwalk virus
        | |||||||   || || || ||| |||||  |||||||||| |||| |||||  ||||||| |
2161 - CTGCCACAAG GATATATCTC ACACCTTGCA AGTGAACAAG CCCCAACTGT TGGTGAGGGT  SRSV/KY/89

6714 - GCCCTGCTCC ACTATGTTGA CCCTGATACC GGTCGGAATC TTGGGGAATT CAAAGCATAC  Norwalk virus
        ||||||||| |||||||||| |||||| ||  |  ||||||| ||||||| ||  || || |||
2221 - CCCCTGCTCC ACTATGTTGA CCCTGACACG GACCGGAATC TTGGGGAGTT TAAGGCTTAC  SRSV/KY/89
```

Figure 12 (6)

```
6774 - CCTGATGGTT TCCTCACTTG TGTCCCCAAT GGGGCTAGCT CGGGTCCACA ACAGCTGCCG  Norwalk virus
       |||||||||| |||| || || |||||| ||| ||||| |||| |||| ||||| ||| || ||
2281 - CCTGATGGTT TCCTAACCTG TGTCCCTAAT GGGGCCAGCT CGGGCCCACA ACAACTACCA  SRSV/KY/89

6834 - ATCAATGGGG TCTTTGTCTT TGTTTCATGG GTGTCCAGAT TTTATCAATT AAAGCCTGTG  Norwalk virus
       |||||||| | |||||||||| |||||||||| |||||||||| ||||||| || ||||||||||
2341 - ATCAATGGAG TCTTTGTCTT TGTTTCATGG GTGTCCAGAT TTTATCAGTT AAAGCCTGTG  SRSV/KY/89

6894 - GGAACTGCCA GCTCGGCAAG AGGTAGGCTT GGTCTGCGCC GATAATGGCC CAAGCCATAA  Norwalk virus
       |||||||||| |  ||||||| |||||||||| ||| |||||| |||||||||  || || ||||
2401 - GGAACTGCCA GTACGGCAAG AGGTAGGCTT GGTTTGCGCC GATAATGGCT CAGGCTATAA  SRSV/KY/89
                                                    ###

6954 - TTGGTGCAAT TGCTGCTTCC ACAGCAGGTA GTGCTCTGGG AGCGGGCATA CAGGTT      Norwalk virus
       |||||||||| ||| || ||  |||||||||| ||||| | ||  || || ||| ||||||
2461 - TTGGTGCAAT TGCCGCCTCT ACAGCAGGTA GTGCTTTAGG GGCAGGTATA CAGGTT      SRSV/KY/89
```

---

* Location of YGDD motif
\+ Location of Primer 35
x Beginning of capsid protein
\# Beginning of third ORF

Parameters for homology search:
- Number of diagonals searched: 25
- Pre-processing ktuple value: 2
- DD algorithm used to find homologies on diagonals (kmatch = 1)
- Scoring matrix used: Unit
- Threshold SD_score for saving homology domains: 3.0
- Minimum length for homology domains: 15

Figure 12 (7)

S_Score: Similarity score of homology domain
SD_Score: (Similarity score - expected score) / std. dev.
Expected similarity score: 4.99
Std. Dev. of similarity scores: 0.43
Threshold SD_score for display of homology domains: 3.0
Equivalent threshold S_Score: 6.3

Comparison:
C1 (1f): |>u 1>+++++ Norwalk virus 58gelcorr8 (7644 bases)+++++>u 7644>|
C2 (1f): |>u 1>+++++ SRSV/KY/89-p36-p3 (2516 bases)+++++>u 2516>|

SD_Score	S_Score	%similar	length	limits_seq_1	limits_seq_2
155.8	72.0	87.2	2516	4494 -> 7009	1 -> 2516

Figure 13a

```
  1 - MMMASKDATS SVDGASASVQ LVPEVNASDP LAMDPVAGSS TAVATAGQVN PIDPWIINNF  SRSV/KY/89
      |||||||||| ||||||   | |||||||||| |||||||||| |||||||||| ||||||||||
  1 - MMMASKDATS SVDGASGAGQ LVPEVNASDP LAMDPVAGSS TAVATAGQVN PIDPWIINNF  Norwalk virus

 61 - VQAPQGEFTI SPNNTPGDVL FDLSLGPHLN PFLLHLSQMY NGWVGNMRVR IMLAGNAFTA  SRSV/KY/89
      |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
 61 - VQAPQGEFTI SPNNTPGDVL FDLSLGPHLN PFLLHLSQMY NGWVGNMRVR IMLAGNAFTA  Norwalk virus

121 - GKIIVSCIPP GFGSQQLTIA QATLFPHVIA DVRTLDPIEV PLEDVRNVLF HNNDRNQQTM  SRSV/KY/89
      |||||||||| ||||* |||| |||||||||| |||||||||| |||||||||| ||||||||||
121 - GKIIVSCIPP GFGSHNLTIA QATLFPHVIA DVRTLDPIEV PLEDVRNVLF HNNDRNQQTM  Norwalk virus

181 - RLVCMLYTPL STGGGTGDSF VVAGRVMTCP SPDFNFLFLV PPTVEQKTRP FTLPNLPLSS  SRSV/KY/89
      ||||||||||  ||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
181 - RLVCMLYTPL RTGGGTGDSF VVAGRVMTCP SPDFNFLFLV PPTVEQKTRP FTLPNLPLSS  Norwalk virus

241 - LSNSRAPLPI SGMGISPDNV QSVQFQNGRC TLDGRLVGTT PVSLSHVAKI RGTSNGTVIN  SRSV/KY/89
      |||||||||| | *|||||||| |||||||||| |||||||||| |||||||||| ||||||||||
241 - LSNSRAPLPI SSIGISPDNV QSVQFQNGRC TLDGRLVGTT PVSLSHVAKI RGTSNGTVIN  Norwalk virus

301 - LTELDGTPFH PFEGPAPIGF PDLGGCDWHI NMTQFGHSSQ TQYDVDTTPD TSVPHLGSIQ  SRSV/KY/89
      |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| | ||||||||
301 - LTELDGTPFH PFEGPAPIGF PDLGGCDWHI NMTQFGHSSQ TQYDVDTTPD TFVPHLGSIQ  Norwalk virus

361 - ANGIGSGNYI GVLSWVSPPS HPSGSQVDLW KIPNYGSSIT EATHLAPSVY SPGFGEVLVF  SRSV/KY/89
      |||||||||* |||||*|||| |||||||||| |||||||||| ||||||||||  |||||||||
361 - ANGIGSGNYV GVLSWISPPS HPSGSQVDLW KIPNYGSSIT EATHLAPSVY PPGFGEVLVF  Norwalk virus

421 - FMSKIPGPGG DSLPCLLPQG YISHLASEQA PTVGEGPLLH YVDPDTDRNL GEFKAYPDGF  SRSV/KY/89
      ||||*||||    |||||||  |||||||||| |||||  ||| |||||| ||| ||||||||||
421 - FMSKMPGPGA YNLPCLLPQE YISHLASEQA PTVGEAALLH YVDPDTGRNL GEFKAYPDGF  Norwalk virus
```

Figure 13a (continued)

```
481 - LTCVPNGASS GPQQLPINGV FVFVSWVSRF YQLKPVGTAS TARGRLGLRR     SRSV/KY/89
      |||||||||| |||||||||| |||||||||| ||||||||||  |||||||||
481 - LTCVPNGASS GPQQLPINGV FVFVSWVSRF YQLKPVGTAS SARGRLGLRR     Norwalk virus
```

```
Parameters for homology search:
    Number of diagonals searched: 25
    Pre-processing ktuple value: 1
    DD algorithm used to find homologies on diagonals (kmatch = 1)
    Scoring matrix used: Dayhoff
    Threshold SD_score for saving homology domains: 3.0
    Minimum length for homology domains: 15

S_Score:  Similarity score of homology domain
SD_Score:  (Similarity score - expected score) / std. dev.
Expected similarity score: 3.14
Std. Dev. of similarity scores:  0.87
Threshold SD_score for display of homology domains:  3.0
Equivalent threshold S_Score: 5.8

Comparison:
P1 : N>u 1>~~~~~~ SRSV/KY/89-p36-p3_853 (531 aa)~~~~~~>u 531>C
P2 : N>u 1>~~~~~~ Norwalk virus-pep-2 (530 aa)~~~~~~>u 530>C
```

SD_Score	S_Score	%similar	length	limits_seq_1	limits_seq_2
51.7	48.1	96.0	530	1 -> 530	1 -> 530

Figure 13b

```
   1 - IEDGPLIYAE HAKYKNHFDA DYTAWDSTQN RQIMTESFSI MSRLTASPEL AEVVAQDLLA   SRSV/KY/89
       |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
1451 - IEDGPLIYAE HAKYKNHFDA DYTAWDSTQN RQIMTESFSI MSRLTASPEL AEVVAQDLLA   Norwalk virus

  61 - PSEMDVGDYV IRVKEGLPSG FPCTSQVNSI NHWIITLCAL SEATGLSPDV VQSMSYFSFY   SRSV/KY/8
       |||||||||| |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
1511 - PSEMDVGDYV IRVKEGLPSG FPCTSQVNSI NHWIITLCAL SEATGLSPDV VQSMSYFSFY   Norwalk virus
                                                                     +

 121 - GDDEIVSTDI DFDPTRLTQI LKEYGLKPTR PDKTEGPIQV RKNVDGLVFL RRTISRDAAG   SRSV/KY/8
       |||||||||| |||| ||||| |||||||||| |||||||||| |||||||||| ||||||||||
1571 - GDDEIVSTDI DFDPARLTQI LKEYGLKPTR PDKTEGPIQV RKNVDGLVFL RRTISRDAAG   Norwalk virus
       +++

 181 - FQGRLDRASI ERQIFWTRGP NHSDPSETLV PHTQRKVQLI SLLGEASLHG EKFYRKISSK   SRSV/KY/8
       |||||||||| |||||||||| |||||||||| ||||||*||| |||||||||| ||||||||||
1631 - FQGRLDRASI ERQIFWTRGP NHSDPSETLV PHTQRKIQLI SLLGEASLHG EKFYRKISSK   Norwalk virus

 241 - VIHEIKTGGL EMYVPGWQAM FRWMRFHDLG LWTGDRNLLP EFVNDD                  SRSV/KY/8
       |||||||||| |||||||||| |||||||||| ||||||*||| ||||||
1691 - VIHEIKTGGL EMYVPGWQAM FRWMRFHDLG LWTGDRDLLP EFVNDD                  Norwalk virus
```

+   Location of YGDD motiff.

Figure 13b (continued)

```
Parameters for homology search:
    Number of diagonals searched: 25
    Pre-processing ktuple value: 1
    DD algorithm used to find homologies on diagonals (kmatch = 1)
    Scoring matrix used: Dayhoff
    Threshold SD_score for saving homology domains: 3.0
    Minimum length for homology domains: 15


S_Score:  Similarity score of homology domain
SD_Score:  (Similarity score - expected score) / std. dev.
Expected similarity score:  3.14
Std. Dev. of similarity scores:  0.87
Threshold SD_score for display of homology domains:  3.0
Equivalent threshold S_Score: 5.8

Comparison:
P1 : N>u 1>~~~~~~ SRSV/KY/89-p36-61_3 (289 aa)~~~~~>u 289>C
P2 : N>u 1>~~~~~~ Norwalk virus-pep-1 (1739 aa)~~~~~>u 1739>C
```

SD_Score	S_Score	%similar	length	limits_seq_1	limits_seq_2
39.4	37.4	99.0	286	1 -> 286	1451 -> 1736

Figure 14a

```
ATGCACTTCA CAGGTGAATA GCATCAACCA CTGGATCCTA    40  SRSV/CDC 6/91
ATGTACCTCA CAAGTGAACA GCATCAATCA CTGGATTTTG    40  SRSV/UT/88
CTGCACATCA CAGTGGAATT CCA-TGCCCA CTGGCTCCTC    39  SMA/78
CTGCACCTCA CAGTGGAACT CCATTGCCCA CTGGTTGCTT    40  SRSV/Cambridge
TTGCACCTCA CAGTGGAACT CCATTGCCCT CTGGTTGCTT    40  SRSV/CDC 32
ATGTACTTCC CAGGTGAACA GCATAAATCA CTGGATAATT    40  NV/8FIIa/68
CTGCACTTCT CAAGTAAATA GCATAAATCA CTGGATAATC    40  SRSV-3/88
CTGCACTTCT CAAGTGAATA GCATAAATCA CTGGATAATC    40  SRSV/KY89/89
 ** ** **  **    **    **     *  **** *  *

ACTCTATGTG CATTGTCAGA AGTCACTGGC TTGTCCCCTG    80  SRSV/CDC 6/91
ACCTTGTGGG GCCTATCAGA AGTTACTGGT CTGGCTCCTG    80  SRSV/UT/88
ACACTCTGTG CACTATCTGA AGTCACAAAC CTGGCTCCTG    79  SMA/78
ACTCTGTGTG CCCTTTCTGA AGTGACAGGA CTAGGCCCCG    80  SRSV/Cambridge
ACTCTGTGTG CCCTTTCTGA AGTGACAGGA CTAGGCCCCG    80  SRSV/CDC 32
ACTCTCTGTG CACTGTCTGA GGCCACTGGT TTATCACCTG    80  NV/8FIIa/68
ACCCTTTGTG CACTGTCTGA GGCTACTGGC TTATCACCTG    80  SRSV-3/88
ACCCTTTGTG CATTGTCTGA GGCTACTGGC TTATCACCTG    80  SRSV/KY89/89
**  * ** *    * ** **  *  **      *    ** *

ATGTGATACA ATCACAATCT TATTTCTCAT TTTATGGT     118  SRSV/CDC 6/91
ATGTAATACA GTCACAATCT TACTTTTCAT TCTATGGT     118  SRSV/UT/88
ACATCATACA AGCTAACTCC TTGTTCTCTT TCTATGGT     117  SMA/78
ACATCATACA AGCTAATTCC ATGTACTCTT TCTATGGT     118  SRSV/Cambridge
ACATCATACA AGCTAATTCC ATGTACTCTT TCTATGGT     118  SRSV/CDC 32
ATGTGGTGCA ATCCATGTCA TATTTCTCAT TTTATGGT     118  NV/8FIIa/68
ATGTGGTGCA GTCCATGTCA TACTTCTCAT TTTACGGT     118  SRSV-3/88
ATGTGGTACA GTCCATGTCA TACTTCTCAT TCTACGGT     118  SRSV/KY89/89
*  *  * **   *    **    *  ** * * **  ***
```

Figure 14b(1)

```
CAATAGAAGATGGCCCCCTCATCTATGCTGAGCATGCTAAATATAAGAATCATTTTGATGCAGATTATAC   70 NV/8FIIa/68
CAATAGAGGATGGCCCTTTAATTTATGCTGAGCATGCCAAGTACAAAAATCATTTTGATGCAGATTACAC   70 SRSV-3/88
CAATAGAGGATGGCCCTTTAATTTATGCTGAACATGCCAAGTACAAAAATCATTTTGATGCAGATTACAC   70 SRSV/KY89
TGTATGAAGATGGTACCATAATATTTGAGAAACATTCCAGATACAGATACCACTATGATGCAGATTATCC   70 SRSV/Cambridge
-GAATGAGGATGGACCCATAATTTTTGAAAAGCACTCCAGGTTCTCATACCACTATGATGCAGATTACTC   69 SMA/78
     ** *****  *  * ** * **   * **  * *  *      * ** * ************  *

AGCATGGG-ACTCAACACAAAATAGACAAATTATGACAGAATCCTTCTCCATTATGTCGCGCCTTACGGC  139 NV/8FIIa/68
AGCATGGG-ACTCTACACAAAATAGACAAATAATGACAGAATCCTTTTCCATCATGTCACGCCTCACGGC  139 SRSV-3/88
AGCATGGG-ACTCTACACAAAATAGACAAATTATGACAGAATCCTTCTCCATCATGTCACGCCTTACGGC  139 SRSV/KY89
-CGCTGGGTACTCCACGCAGCAACGGGCAGTGTTGGCAGCAGCACTTGAAATCATGGTGAGGTTCTCTGC  139 SRSV/Cambridge
ACGCTGGG-ACTCAACCCAACAGAGGGCAGTGCTAGCTGCAGCCTTGGAAATCATGGTAAAATTCTCACC  138 SMA/78
    **** **** ** **  *  *   * *  *  * * * *  *    ** ***       *  *  *

CTCACCAGAATTGGCCGAGGTTGTGGCCCAAGATTTGCTAGCACCATCTGAGATGGATGTAGGTGATTAT  209 NV/8FIIa/68
CTCTCCAGAACTAGCTGAGGTTGTAGCCCAGGACTTGCTAGCACCATCCGAGATGGATGTGGGTGACTAT  209 SRSV-3/88
CTCTCCAGAACTAGCTGAGGTTGTAGCCCAGGACTTACTAGCACCATCCGAGATGGATGTGGGCGACTAT  209 SRSV/KY89
TGAACCACAGCTAGCGCAAATAGTAGCTGAAGATCTGCTAGCACCAAGTGTAGTTGATGTGGGTGACTTC  209 SRSV/Cambridge
AGAACCACATTTGGCCCAAATTGTTGCAGAGGATCTCCTAGCCCCCAGTGTGATGGATGTAGGTGATTTC  208 SMA/78
    *** *  * **  *  * ** **  * **  * ***** **    *   * ***** ** ** *
```

Figure 14b(2)

```
GTCATCAGGGTCAAAGAGGGGCTGCCATCTGGATTCCCATGTACTTCCCAGGTGAACAGCATAAATCACT  279 NV/8FIIa/68
GTTATAAGGGTCAAAGAAGGCCTACCATCAGGATTTCCCTGCACTTCTCAAGTAAATAGCATAAATCACT  279 SRSV-3/88
GTTATAAGGGTCAAAGAAGGCCTACCATCAGGATTTCCCTGCACTTCTCAAGTGAATAGCATAAATCACT  279 SRSV/KY89
AAGATCACCATTAATGAAGGCCTACCTTCTGGTGTGCCCTGCACCTCACAGTGGAACTCCATTGCCCACT  279 SRSV/Cambridge
AAAATAACAATTAATGAGGGACTGCCCTCGGGAGTACCCTGCACATCACAGTGGAATTCCAT-GCCCACT  277 SMA/78
   ** *    * ** ** ** ** ** ** **  * ** ** ** ** **    **   ***    ****

GGATAATTACTCTCTGTGCACTGTCTGAGGCCACTGGTTTATCACCTGATGTGGTGCAATCCATGTCATA 349 NV/8FIIa/68
GGATAATCACCCTTTGTGCACTGTCTGAGGCTACTGGCTTATCACCTGATGTGGTGCAGTCCATGTCATA 349 SRSV-3/88

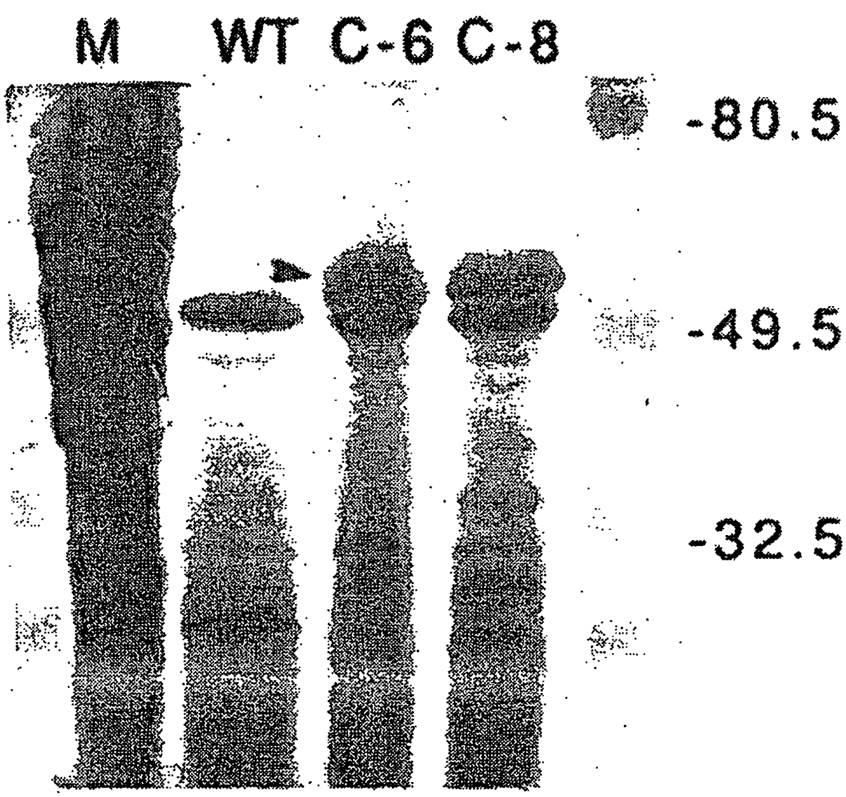
GGATAATCACCCTTTGTGCATTGTCTGAGGCTACTGGCTTATCACCTGATGTGGTACAGTCCATGTCATA 349 SRSV/KY89
GGTTGCTTACTCTGTGTGCCCTTTCTGAAGTGACAGGACTAGGCCCCGACATCATACAAGCTAATTCCAT 349 SRSV/Cambridge
GGCTCCTCACACTCTGTGCACTATCTGAAGTCACAAACCTGGCTCCTGACATCATACAAGCTAACTCCTT 347 SMA/78
** *  * ** ** *****  * ***** *  **      *     ** **  *  * **  * *  **

TTTCTCATTTTATGGTGATGATGAGATTGTGTCAACTGACATAGATTTTGACCCAGCCCGCCTCACTCAA  419 NV/8FIIa/68
CTTCTCATTTTACGGTGATGATGAGATTGTGTCAACTGACATAGACTTTGATCCAACTCGACTCACCCAA  419 SRSV-3/88
CTTCTCATTCTACGGTGATGATGAGATCGTATCAACTGACATAGACTTTGACCCAACTCGCCTCACCCAA  419 SRSV/KY89
GTACTCTTTCTATGGTGATGATGAGATTGTGAGTACTGACATAAAATTGGACCCAGAGAAACTGACTGCA 419 SRSV/Cambridge
GTTCTCTTTCTATGGTGATGATGAAATCGTAAGTACTGACATAAAATTAGACCCAGAGAAACTCACAGCA  417 SMA/78
 * *** ** ** *********** ** **     ********* * ** ** ***       ** **   *
```

Figure 14b(3)

```
ATTCTCAAGGAA  431 NV/8FIIa/68
ATCCTCAAGGAA  431 SRSV-3/88
ATTCTCAAGGAA  431 SRSV/KY89
AAACTCAAAGA-  430 SRSV/Cambridge
AAACTC------  423 SMA/78
*   ***
```

Figure 15



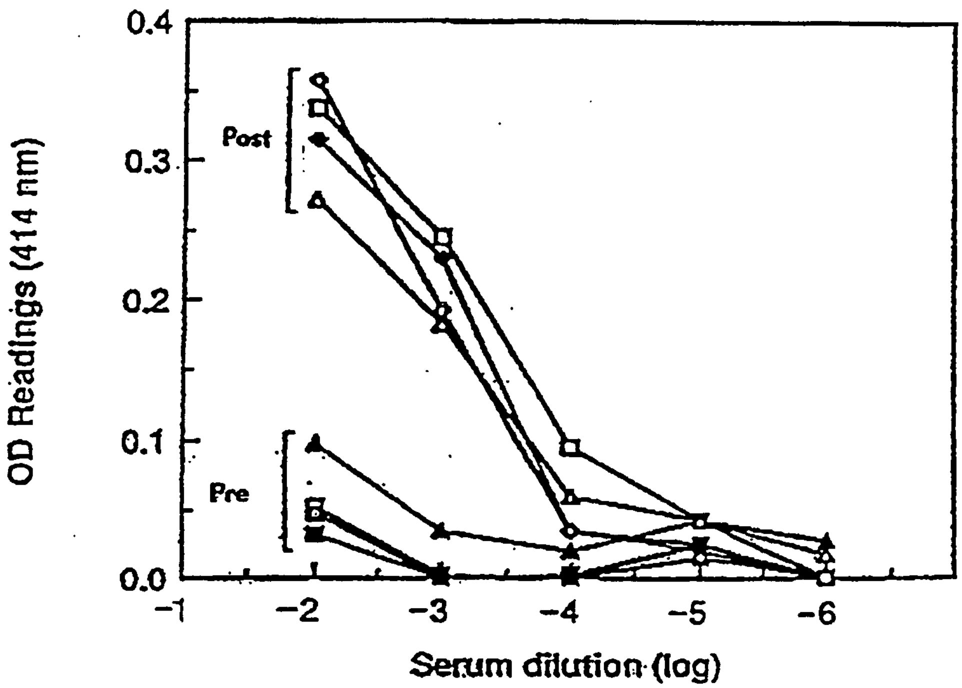
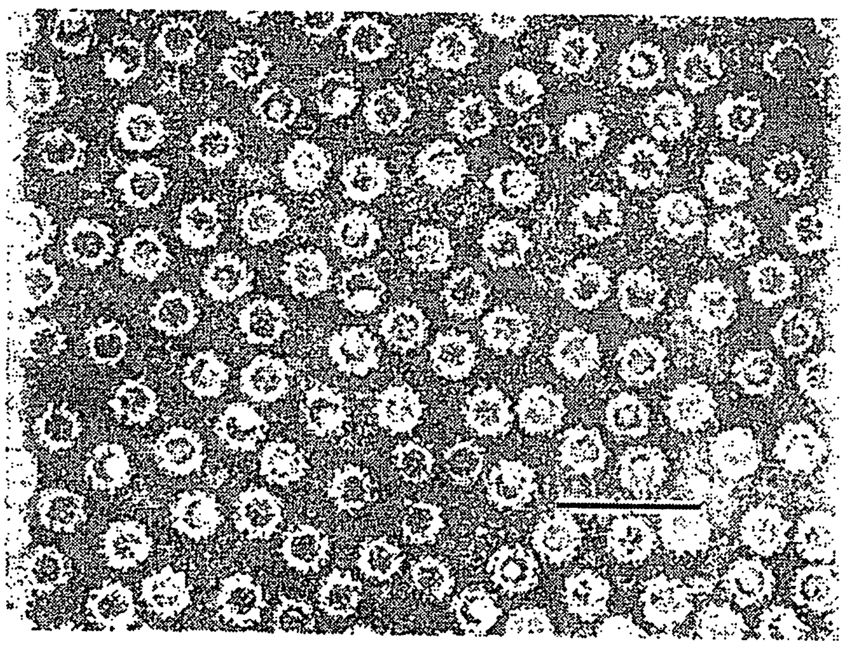

0.4
0.3
0.2
0.1
0.0
OD Readings (414 nm)
Post
Pre
−1
−2
−3
−4
−5
−6
Serum dilution (log)

FIG 16

Figure 17



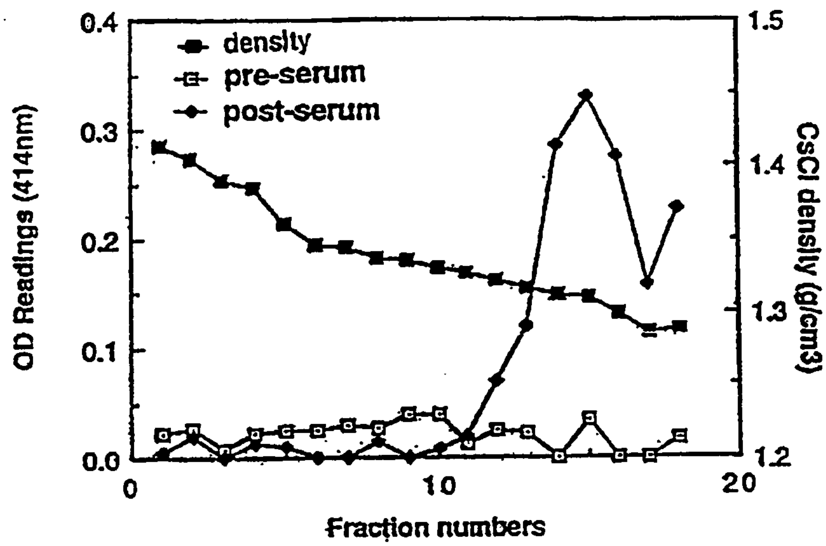

0.4
0.3
0.2
0.1
0.0
1.5
1.4
1.3
1.2
0
10
20
density
pre-serum
post-serum
OD Readings (414nm)
CsCl density (g/cm3)
Fraction numbers

FIG 18

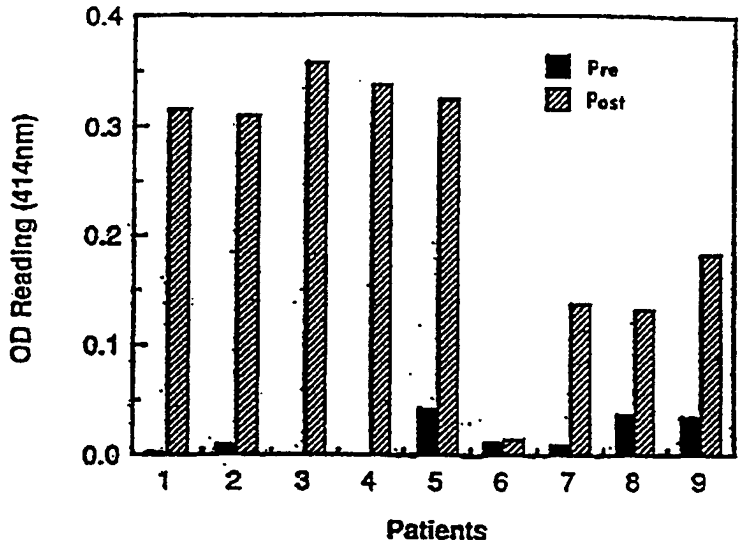


FIG 19

```
TGGACGGACC  TGCTGTTGAA  GATCTCTTCA
AANGGCTCGA  ACGACCAAAG  CACGATCGGT
ATTGTGTTGA  CTACGCAAAG  TGGGACTCAA
CCCANCCACC AAAAGTAACA TCCAATCAAT
NGACATC....
GTGANATGNN  ACATCTTCGA  CTCGATGGAC
CTATTCACAT  ATGGTGATGA  CGGTGTCTAC
ATCGTCCCAC  CACTATATCA  TCTGTCATGC
CCAAGTCTTC ACCAACCTGA AAC
```

FIG 20